From transforming growth factor-β signaling to androgen action: Identification of Smad3 as an androgen receptor coregulator in prostate cancer cells

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Although transforming growth factor-β (TGF-β) has been identified to mainly inhibit cell growth, the correlation of elevated TGF-β with increasing serum prostate-specific antigen (PSA) levels in metastatic stages of prostate cancer has also been well documented. The molecular mechanism for these two contrasting effects of TGF-β, however, remains unclear. Here we report that Smad3, a downstream mediator of the TGF-β signaling pathway, functions as a coregulator to enhance androgen receptor (AR)-mediated transactivation. Compared with the wild-type AR, Smad3 acts as a strong coregulator in the presence of 1 nM 5α-dihydrotestosterone, 10 nM 17β-estradiol, or 1 μM hydroxyflutamide for the LNCaP mutant AR (mtAR T877A), found in many prostate tumor patients. We further showed that endogenous PSA expression in LNCaP cells can be induced by 5α-dihydrotestosterone, and the addition of the Smad3 further induces PSA expression. Together, our findings establish Smad3 as an important coregulator for the androgen-signaling pathway and provide a possible explanation for the positive role of TGF-β in androgen-promoted prostate cancer growth.

Androgen action controls the development and proper functioning of the male reproductive system, including the prostate and the epididymis (1), as well as many nonreproductive systems, such as muscle, skin, hair follicles, and the brain. The androgen receptor (AR), a member of the steroid receptor superfamily, functions as an androgen-dependent transcriptional regulator (2). After binding to ligand, the activated AR is able to recognize palindromic DNA sequences, called androgen response elements (AREs), and form a complex with AR-associated proteins to induce the expression of AR target genes. Several AR coregulators, androgen receptor-associated proteins (ARAs) such as ARA24, ARA54, ARA55, ARA70, ARA160, Rb, and TIFIIH, have been isolated and characterized (3–10). Results from these studies suggest that coregulators not only can enhance AR transactivation, but may also be able to increase the agonist activity of antiandrogens and 17-β estradiol (E2) in prostate cancer DU145 cells.

Transforming growth factor β (TGF-β) signaling is mediated through two types of transmembrane serine/threonine kinase receptors (11). Upon binding to TGF-β, the type II TGF-β receptor (TβRII) forms a heteromeric complex with the type I TGF-β receptor (TβRI), resulting in the phosphorylation and activation of TβRI (12). The activated TβRI then interacts with an adaptor protein SARA (Smad anchor for receptor activation) (13), which propagates signals to intracellular signaling mediators known as Smad2 and Smad3 (14). After association with Smad4, the Smad complexes translocate to the nucleus, where they activate specific target genes through cooperative interactions with DNA and other DNA-binding proteins such as FAST1 and Fos/Jun (AP-1) (15, 16).

TGF-β plays a dual role in tumorigenesis. On the one hand, TGF-β inhibits the growth of normal epithelial and endothelial cells (17) and induces cell-cycle inhibitors such as $p15^{INK4B}$ and $p21^{WAF1/CIP}$ (18, 19). On the other hand, TGF-β can accelerate the malignant process during late stages of tumorigenesis (20, 21). TGF-β is abundantly expressed in various tumors of epithelial origin (22) in which it can suppress immune surveillance (23), facilitate tumor invasion (21), and promote the development of metastases (24). The study of TGF-β expression indicates that it may be involved in the development of prostate cancer in animal models (25). Moreover, plasma TGF-β was significantly elevated in patients with clinically evident metastases and correlated with increasing serum prostate-specific antigen (PSA) levels (26, 27). The detailed mechanism for the relationship between TGF-β signaling and PSA in prostate carcinogenesis, however, remains unclear. Here we report that TGF-β can enhance AR-mediated transactivation via the interaction of AR and Smad3 in two prostate cancer cell lines, DU145 and PC-3. We further showed that endogenous PSA expression in LNCaP cells can be induced by 5α-dihydrotestosterone (DHT), and the addition of the Smad3 further induces the PSA expression levels. These findings provide linkage between two signaling pathways at the levels of androgen-AR and TGF-β/Smad3, which may play an important role in prostate tumorigenesis.

Materials and Methods

Chemicals and Plasmids. DHT, dexamethasone, progesterone, and E2 were obtained from Sigma, and hydroxyflutamide (HF) was from Schering. pSG5 wild-type AR (wtAR), pCMV-AR, and pCMV-mtARt877a (mutant AR derived from the prostate cancers, codon 877 mutation threonine to alanine) were used in our previous report (4). Expression plasmids for glutathione *S*-transferase (GST)-Smad3 and full-length cDNAs of human Smad3 were kindly provided by Rik Derynck (Univ. of California, San Francisco) (28). TβRI, TβRII receptors and constitutively active TGF-β type I receptor (TβRI-T204D) expression vectors were provided by Jeffery L. Wrana (Univ. of Toronto) (12).

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Abbreviations: AR, androgen receptor; TGF-β, transforming growth factor β; TβRI, TGF-β receptor type I; TβRII, TGF-β receptor type II; wtAR, wild-type androgen receptor; mtAR, mutant AR; ARA, androgen receptor associated protein; DHT, 5α-dihydrotestosterone; E2, 17β-estradiol; HF, hydroxyflutamide; DBD, DNA-binding domain; LBD, ligand-binding domain; PSA, prostate-specific antigen; CAT, chloramphenicol acetyltransferase; GST, glutathione *S*-transferase; VDR, vitamin D receptor; ARE, androgen response element; MMTV, mouse mammary tumor virus.

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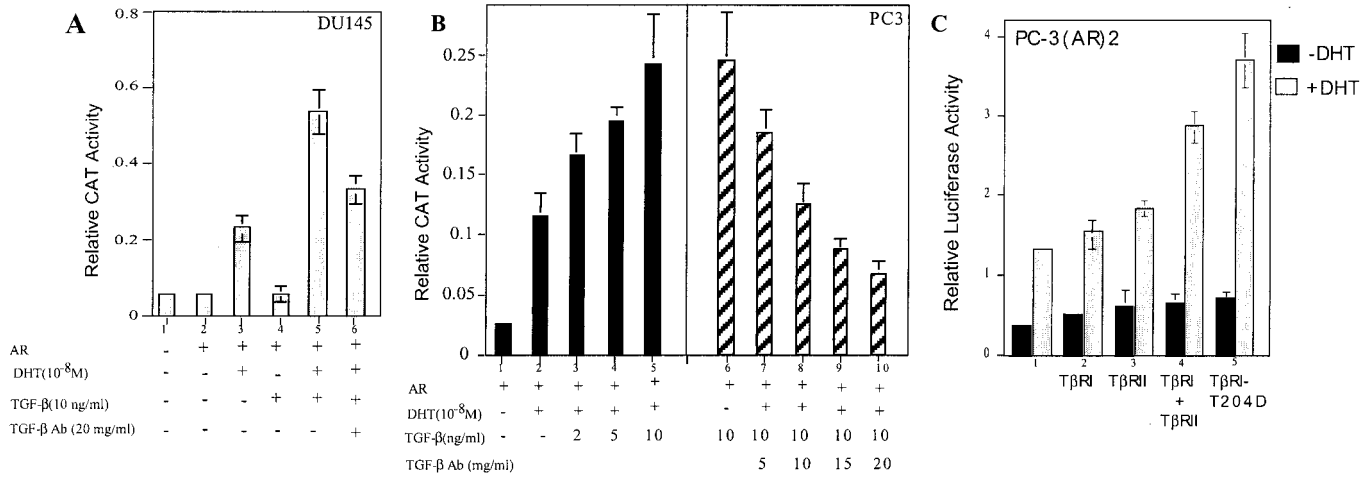

Fig. 1. The ligand-induced transactivation of AR is enhanced by treatment with TGF-β. (*A*) CAT assays were performed with extracts from DU145 cells transfected with AR expression vector (pSG5-AR) (1 μg) in the presence (+) or absence (−) of DHT (10^{-8} M) or TGF-β1 (10 ng/ml) or specific TGF-β1 neutralizing antibody (20 mg/ml). (*B*) (*Left*) PC-3 cells were transfected with pSG5-AR (1 μg) in the presence (+) or absence (−) of DHT (10^{-8} M) with increasing amounts of TGF-β1. (*Right*) A fixed amount of TGF-β1 (10 ng/ml) was added to transfected PC-3 cells with increasing amounts of specific TGF-β1 neutralizing antibody. (*C*) PC-3(AR)2 cells stably transfected with AR were overexpressed with TGF-β type I (TβRI) or type II (TβRII) receptor or constitutively active TGF-β type I receptor (TβRI-T204D) as indicated. Three micrograms of MMTV-CAT or MMTV-Luc was used as a reporter plasmid in all experiments. All values represent the averages ± SD of four independent experiments.

Cell Culture and Transfections. Human prostate cancer DU145 cells and PC-3 cells were maintained in DMEM containing penicillin (25 units/ml), streptomycin (25 μg/ml), and 5% FCS. Transfections were performed with the calcium phosphate precipitation method, and cells were harvested after 24 h for the chloramphenicol acetyltransferase (CAT) assay, as described previously (5). The CAT activity was visualized and quantitated with STORM 840 (Molecular Dynamics). At least three independent experiments were carried out in each case. The SW480.7 cells and PC3 (AR)2 cells are gifts from Eric J. Stanbridge and T. J. Brown (Univ. of California, Irvine).

GST Pull-Down Assay. Fusion proteins of GST-Smad3 and GST-AR, and GST protein alone were obtained by transforming expressing plasmids into BL21 (DE3) pLysS strain-competent cells followed with 1 mM isopropyl β-D-thiogalactoside induction. GST fusion proteins then were purified by glutathione-Sepharose 4B (Amersham Pharmacia). The AR and Smad3 proteins labeled with ^{35}S were generated *in vitro* with the TNT-coupled reticulocyte lysate system (Promega). For the *in vitro* interaction, the glutathione-Sepharose bound GST proteins were mixed with 5 μl of ^{35}S-labeled TNT proteins in the presence or absence of 1 μM DHT at 4°C for 3 h. The bound proteins were separated on an 8% SDS-polyacrylamide gel and visualized by PhosphorImager (Molecular Dynamics).

Mammalian Two-Hybrid Assay. The mammalian two-hybrid system mainly followed the protocol of CLONTECH, with some modifications. Human prostate cancer DU145 cells were transiently cotransfected with Gal4-Smad3 expression plasmid, VP16-AR expression plasmid, and pG5CAT reporter plasmid in the presence or absence of 10 nM DHT. CAT assays were performed as described above.

Coimmunoprecipitation of AR and Smads. PC-3 cells were cotransfected with AR and FLAG-Smad3 for 16 h and then treated with vehicle or 10 nM DHT for another 16 h. PC3(AR)2 cells were treated with vehicle or 10 nM DHT for16 h. The cells were lysed and incubated with monoclonal anti-FLAG antibody (Sigma), polyclonal Smad3 antibody (Santa Cruz Biotechnology), or control IgG at 4°C for 2 h, depending on the experimental design, followed by the addition of protein A/G beads (Santa Cruz Biotechnology) for 1 h at 4°C. The bound proteins were separated on an 8% SDS-polyacrylamide gel and blotted with polyclonal AR antibody (NH27), Smad3 antibody, or anti-FLAG antibody. The bands were detected with an alkaline phosphatase detection kit (Bio-Rad).

Northern Blot Analysis. The blot containing approximately 20 μg of total RNA from LNCaP cells was transfected with Smad3 for 16 h, followed by DHT treatment for another 16 h. PSA expression level was determined by hybridizing with a probe from exon 1 of the PSA gene and labeled with [α-^{32}P]dCTP. A β-actin probe was used as a control for equivalent RNA loading.

Results

Enhancement of AR-Mediated Transactivation by TGF-β in Different Prostate Cancer Cells. To study the potential correlation between androgen and TGF-β in prostate cancer cells, we first choose TGF-β-responsive prostate cancer DU145 and PC-3 cells to examine the effect of TGF-β on androgen-induced mouse mammary tumor virus (MMTV) promoter activity. Activation of MMTV-CAT activity was achieved by transient transfection of AR in the presence of 10^{-8} M DHT (Fig. 1*A*, Lanes 1–3), and this AR-mediated transactivation was enhanced by the addition of TGF-β in DU145 cells (Fig. 1*A*, Lane 3 vs. Lane 5). Furthermore, this induction was partially blocked by adding TGF-β-specific neutralizing antibody (Fig. 1*A*, Lane 5 vs. Lane 6). Similar results were obtained with PC-3 cells, where AR-mediated transactivation was enhanced by TGF-β (Fig. 1*B*, Lane 2 vs. Lanes 3–5) and suppressed by the TGF-β-specific neutralizing antibody (Fig. 1*B*, Lane 6 vs. Lanes 7–10), both in a dose-dependent manner (Fig. 1*B*). Because Western blot analysis indicated that PC-3 cells stably transfected with AR, PC-3(AR)2, express similar amounts of AR as compared with LNCaP cells and the increased AR-mediated transactivation by TGF-β did not change the expression level of AR (data not shown), we further examined the effect of TGF-β receptors in PC-3(AR)2. As shown in Fig. 1*C*, in the presence or absence of androgen, TβRI or TβRII receptor alone has a marginal effect

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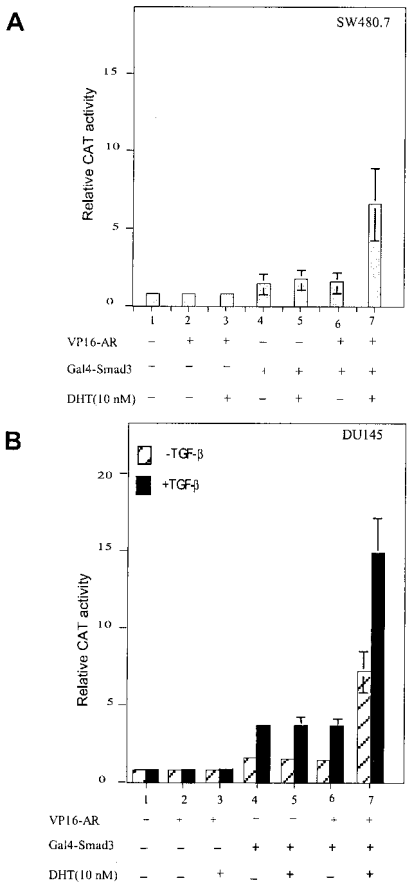

Fig. 2. The association of Smad3 with AR in a mammalian two-hybrid interaction system. (*A*) SW480.7 cells were cotransfected with 3 μg of Gal4-Smad3 encoding the full-length Smad3 fused to the Gal4-DBD and 4.5 μg of VP16-AR encoding the full-length AR fused to the activation domain of VP16. Interaction was estimated by determining the level of CAT activity from 3 μg of the reporter plasmid pG5-CAT in the presence of 10^{-8} M DHT. (*B*) DU145 cells were transfected with Gal4-Smad3 and VP16-AR expression vectors in the presence (+) or absence (−) of DHT and TGF-β. Each CAT activity is presented relative to the transactivation observed in the absence of DHT. All values represent the mean ± SD of four independent experiments.

on AR-mediated transactivation. However, coexpression of TβRI and TβRII receptor or constitutively active TGF-β type I receptor (TβRI-T204D) could further enhance AR transactivation in the presence of DHT. Taken together, these data suggest that TGF-β may be able to cross-talk with the androgen/AR pathway without altering the expression of AR.

Association Between AR and Smad3 *in Vitro* and *in Vivo*. Next, we examined the possibility of interaction between AR and Smad3, the mediator of TGF-β signaling. We first applied the mammalian two-hybrid assay in SW480.7 cells that lack Smad4 but still express Smad1 and Smad3 (29). The results show that DHT, at concentrations greater than 1 nM, promotes the interaction between Smad3 and AR (Fig. 2*A*, lane 7), indicating that Smad3 is sufficient to interact with AR. To further explore the mechanism underlying this association between AR and Smad3, we treated prostate DU145 cells with TGF-β to determine whether TGF-β was involved. As shown in Fig. 2*B*, transient transfection of either Gal4-Smad3 or VP16-AR alone showed negligible activity (lanes 2–5). The CAT activity was induced when VP16-AR was coexpressed with Gal4-Smad3 in the presence of 10 nM DHT (lane 7, hatched bar). Upon TGF-β stimulation the reporter gene activity was further induced (lane 7, solid bar); however, TGF-β cannot exert this effect in the absence of DHT (lane 6). These results indicate that the association between AR and Smad3 is an androgen-dependent process, and TGF-β can further enhance this interaction.

To further demonstrate the interaction between AR and Smad3, N-terminal Flag-tagged, full-length Smad3 was expressed in PC-3 cells alone or cotransfected with wtAR. Cell extracts were prepared and immunoprecipitations were performed with the use of anti-Flag antibodies, followed by Western blotting with anti-AR antibodies. In the presence of Flag-Smad3, AR was coimmunoprecipitated with Smad3 in both the presence or absence of 10 nM DHT (Fig. 3*A*). Next, an *in vivo* coimmunoprecipitation assay was applied to demonstrate that the endogenous Smad3 is capable of interacting with AR. As shown in Fig. 3*B*, AR was detected in the Smad3 immunocomplex in the absence or presence of androgen in PC-3(AR)2 but not in PC-3 cells. A similar result was also obtained when we replaced PC-3(AR)2 with LNCaP cells (data not shown).

To determine which individual domain of AR can interact with Smad3, we used GST-Smad3 fusion proteins incubated with

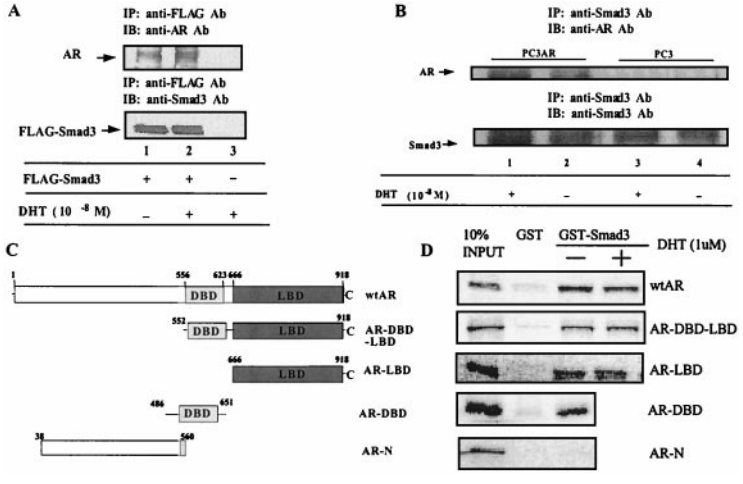

Fig. 3. *In vivo* and *in vitro* interaction between Smads and AR. (*A* and *B*) Coimmunoprecipitation of AR and Smad3. (*A*) PC-3 cells that overexpressed Flag-Smad3 and AR. (*B*) PC-3 and PC-3(AR)2 cells were treated with or without DHT. Cell extracts were prepared and immunoprecipitations were performed with the use of anti-FLAG antibody or anti-Smad3 antibody, followed by immunoblotting with antibody to AR. (*C*) The wtAR and different AR deletion mutants used in the GST pull-down assay are shown schematically. (*D*) Interaction domains of AR for Smad3. A series of ^{35}S-labeled mtARs incubated with GST-Smad3 or GST alone in the presence (+) or absence (−) of 1 μM DHT were tested for interaction in the GST pull-down assay.

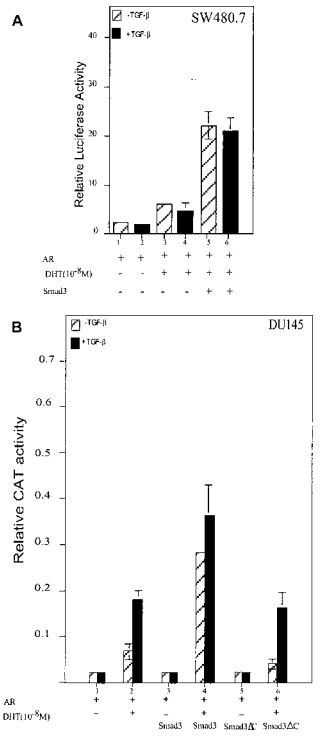

Fig. 4. The effects of Smad3 on AR-mediated transcriptional activity. (*A*) SW480.7 cells were cotransfected with 1 μg of pSG5-AR, 3 μg of MMTV-CAT, and 3 μg of Smad3 expression vectors in the presence (+) or absence (−) of DHT (10^{-8} M) or TGF-β (10 ng/ml). (*B*) DU145 cells were cotransfected with 3 μg of Smad3 or Smad3ΔC mutant expression vectors with 1 μg of pSG5-AR and 3 μg of MMTV-CAT, in the presence (+) or absence (−) of DHT (10^{-8} M) or TGF-β (10 ng/ml). Each CAT activity is presented relative to the transactivation observed in the absence of DHT, and an error bar represents the mean ± SD of four independent experiments.

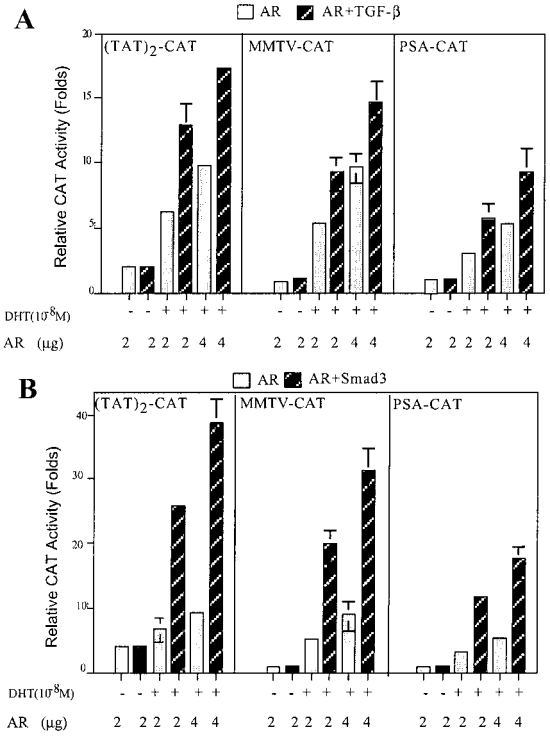

Fig. 5. The androgen response element is important for TGF-β/Smad3-enhanced AR transactivation. (*A*) DU145 cells were transiently cotransfected with AR (2 or 4 μg) and tyrosine aminotransferase–CAT, MMTV-CAT, or PSA-CAT (3 μg), in the presence (+) or absence (−) of DHT (10^{-8} M) or TGF-β (10 ng/ml). (*B*) DU145 cells were transiently cotransfected with AR (2 or 4 μg) and tyrosine aminotransferase–CAT, MMTV-CAT, or PSA-CAT (3 μg), and Smad3 expression vector (6 or 10 μg) in the presence (+) or absence (−) of DHT (10^{-8} M). Each CAT activity is presented relative to the transactivation observed in the absence of Smad3. All values represent the mean ± SD of three independent experiments.

various AR deletion mutants (shown in Fig. 3*C*) in pull-down experiments (Fig. 3*D*). The full-length wtAR could interact with Smad3 in the presence and absence of 1 μM DHT. Whereas DNA-binding domain (DBD)-LBD AR peptides could interact with Smad3, we found that both DBD AR and LBD AR peptides interacted with Smad3 but N-terminal AR peptide failed to interact with Smad3. Furthermore, two AR mutants (mtAR R614H with a mutation at the second zinc finger of the DBD and mtAR E708K with mutation at the LBD) were still able to interact with Smad3 (data not shown). These results suggest that AR may contain two independent binding sites located in both DBD and LBD domains to interact with Smad3.

Roles of Smad3 in AR-Mediated Transactivation. Next, we attempted to determine whether Smad3 can enhance androgen-induced AR transactivation in SW480.7 cells that are unresponsive to the inhibitory effects of TGF-β. As shown in Fig. 4*A*, Smad3 increased the ligand-dependent transactivation of AR, suggesting that Smad3 was able to function as a positive AR coregulator to enhance AR transactivation. Similarly, the enhanced transactivation function of AR by Smad3 was observed in DU145 cells (Fig. 4*B*). A C-terminal deletion of 39 amino acids resulted in the loss of the Smad3-enhanced effect of the MMTV-CAT reporter gene in DU145 cells. As previous reports showed that the MH2 region of the C-terminal Smad3 is essential for homooligomerization and heterooligomerization (11), it is possible that this region is also important for Smad3 to interact with AR and exert its function as an active coregulator for AR.

ARE Is Important for TGF-β/Smad3-Enhanced AR Transactivation. To test whether the ARE is important for TGF-β and Smad3 to enhance AR-mediated transactivation, DU145 cells were transiently transfected with MMTV and PSA, two of the AR target natural promoters, and one synthetic promoter, tyrosine aminotransferase, which contains only two copies of a synthetic ARE. As shown in Fig. 5, increasing AR led to a higher degree of transactivation in a DHT-dependent manner, and TGF-β and Smad3 were able to further enhance both the natural and synthetic ARE promoters.

To rule out any indirect effects on the basal activity of the MMTV-ARE CAT reporter, we also removed the ARE DNA fragment from our reporter (MMTV-ΔARE-CAT). The results showed that TGF-β and Smad3 could not induce any activity (data not shown). Taken together, these results suggest that the ARE is essential for TGF-β/Smad3 to exert their influence on AR transactivation.

Effect of Smad3 on the Transactivation of the Progesterone Receptor (PR), Vitamin D Receptor (VDR), Estrogen Receptor (ER), wtAR, and mtAR. Several identified coregulators, such as SRC-1 (30), CBP/p300 (31), and GRIP1/TIF2 (32, 33), enhance the transactivation of most steroid receptors. It is therefore important to investigate whether Smad3 can function as a general coregulator for other steroid receptors through their cognate ligands and response elements in DU145 cells. Among all of the classic steroid receptors we tested, Smad3 could significantly enhance the transactivation of AR, PR, and VDR (Fig. 6*A*). These data

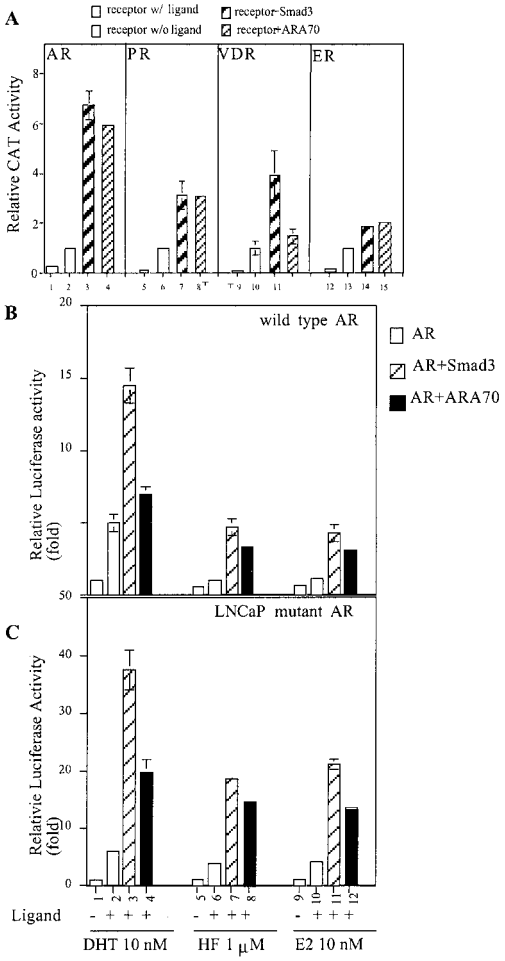

Fig. 6. Effect of Smad3 on the transcriptional activities of wtAR, mtAR, progesterone receptor (PR), VDR, and estrogen receptor (ER). (*A*) DU145 cells were transiently cotransfected with 3 μg of reporter plasmids (MMTV-CAT for AR and PR, ERE-CAT for ER, and VDRE-CAT for VDR), 1 μg of each receptor constructed in pSG5, and 4.5 μg of Smad3 expression vector in the presence of 10^{-8} M of each cognate ligand. Each luciferase and CAT activity is presented relative to the transactivation observed in the absence of Smad3. (*B*) 1.5 μg of wtAR was cotransfected with 4.5 μg of Smad3 or ARA70 in the absence or presence of DHT, E2, or HF at indicated concentrations. (*C*) The LNCaP mtART877a was used to replace the wtAR to perform the same experiment as in *B*. All values represent the mean ± SD of three independent experiments.

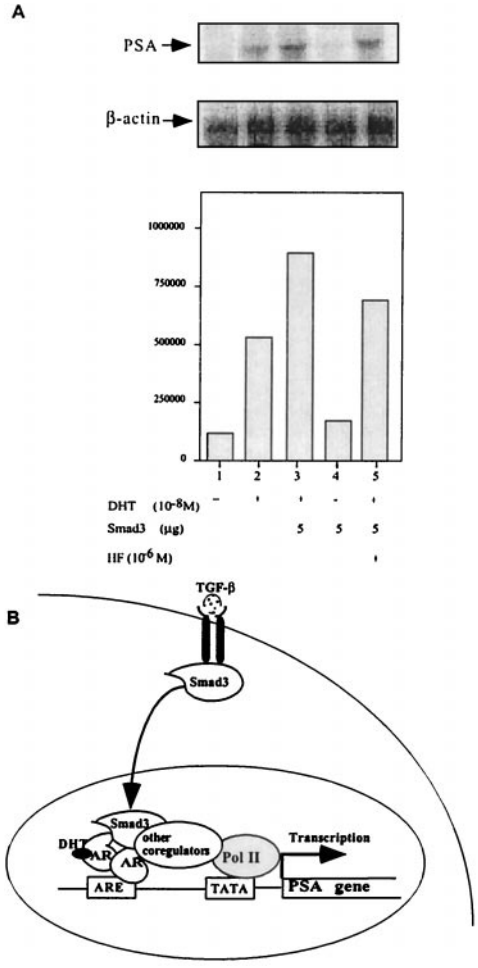

Fig. 7. AR-induced PSA expression is potentiated by Smad3. (*A*) Smad3-enhanced androgen/AR-induced PSA mRNA expression. LNCaP cells were transfected with Smad3 and parent vector as indicated for 16 h, followed by DHT treatment for another 16 h. The PSA expression level was determined by Northern blotting. The probe was obtained from exon 1 of the PSA gene and labeled with [α-^{32}P]dCTP. A β-actin probe was used as a control for equivalent mRNA loading. (*B*) A model for androgen and TGF-β pathways in AR-mediated PSA transcription.

are also in agreement with the previous report showing that Smad3 can interact with VDR and enhance VDR target genes (34). Because the androgen signal pathway is the opposite of the vitamin D signal pathway in the modulation of prostate cell growth, identification of Smad3 as an AR-positive coregulator may provide a possible explanation for TGF-β signals in androgen-mediated prostate cancer cell growth.

One of the popular explanations of how prostate cancer progresses from an androgen-dependent to an androgen-independent stage is that mutations in AR may change the specificity and sensitivity of AR to antiandrogens, such as HF (9). Thus, it is in our interest to investigate whether Smad3 can enhance the agonist activity of these antiandrogens on wtAR and mtARs. Results from PC3 cells show that wtAR responded well to DHT at 10 nM, and Smad3 enhanced this transactivation by another 3- to 4-fold (Fig. 6*B*). On the other hand, wtAR was only able to respond marginally to 1 μM HF and 10 nM E2, but Smad3 could further promote the wtAR transactivation in the presence of 1 μM HF and 10 nM E2. We further extended these findings to the AR mutant mtART877a, which is found in many prostate tumors and LNCaP cells (35). Previous reports showed that LNCaP mtART877a could be stimulated by E2, progesterone, and flutamide (35). In comparison, our data showed that mtART877a responded much better to HF and E2 than did wtAR (Fig. 6*C*). Furthermore, Smad3 could promote this E2- or HF-mediated androgenic activity on mtART877a. Compared with the previously identified coregulator, ARA70, Smad3 showed a relatively stronger enhancement effect on the AR transactivation. Together, these results suggest that the LNCaP AR may require Smad3 for proper or maximal DHT-, E2-, or HF-mediated transactivation.

AR-Induced PSA Expression Is Enhanced by Smad3. A previous study reported that plasma TGF-β was significantly elevated in patients with clinically evident prostate metastases and correlated with PSA levels (26, 27). Therefore, it is important to investigate the effect of Smad3 on androgen-induced PSA expression to understand the mechanism of prostate carcinoma progression. As shown in Fig. 5, increasing AR induced PSA reporter gene activity in a DHT-dependent manner, and TGF-β or Smad3 was able to further enhance PSA promoter activity. Our Northern blot data show that endogenous PSA expression in LNCaP cells

can also be induced by DHT. Addition of Smad3 can further enhance PSA expression in the presence of androgen (Fig. 7*A*, lane 2 vs. lane 3). As a control, our data also demonstrated that addition of Smad3 failed to induce PSA expression in the absence of androgen (Fig. 7*A*, lane 1 vs. lane 4). Furthermore, this Smad3-enhanced PSA induction can be partially repressed by HF, suggesting that Smad3 may play positive roles in enhancing PSA expression via cooperation with AR in the presence of androgen.

Discussion

In this study, we have investigated the mechanism of induction of androgen signaling by the TGF-β pathway in prostate cancer cells. Fig. 7*B* shows a model for TGF-β enhanced AR-mediated transactivation, with the androgen-inducible ARE segment representing the entire PSA promoter. First, TGF-β-enhanced AR transactivation may go through Smad3 as a positive coregulator. As Smad3 can interact and enhance AR in Smad4-deficient cells, it is likely that Smad3, without heterodimerizing with Smad4, should be able to enhance AR transactivation in response to androgens and TGF-β. However, we do not know whether Smad3 may bind directly to PSA promoter, nor do we know the stoichiometry of the AR/Smad3 complex for the maximal induction. Second, after AR binding to the ARE, the AR/Smad3 complex likely recruits transcription adaptors and other coregulators, leading to enhanced transcription of the PSA gene. As noted, other transcription factors may also bind to AR and/or the promoter of the PSA gene to induce AR transactivation. This model suggests a critical role for TGF-β in enhancing the interaction between Smad3 and AR to induce AR transactivation.

AR acts synergistically or antagonistically with a number of signaling pathways. Previously, evidence emerged indicating that the steroid receptors can down-regulate the expression of certain genes by interfering with the function of other transcription factors. AR interference with members of the AP-1 transcription factor family is well documented (36). On the other hand, overexpression of AP-1 also repressed androgen-induced PSA promoter activity (37). This mutual inhibition with heterologous transcription factors has been reported to involve either direct protein–protein contacts or competition for limiting amounts of common coregulators. Previous studies have shown that the AP-1 complex can bind directly to Smad3, which is required for the activation of AP-1 elements. Here we provide evidence supporting a role for Smad3 as a coregulator for AR, in addition to its role as a TGF-β transcription mediator. If the cellular concentration of Smad3 is limited in cells, we would expect to observe AR overexpression to interfere with AP-1-mediated transcription by competing for Smad3.

One of the physiological functions of TGF-β is to restrain the proliferation of normal epithelial, endothelial, and hematopoietic cells, thus contributing to the maintenance of homeostasis in these tissues (17). This function of TGF-β is often lost in cancer as a result of mutations that directly inactivate components of the TGF-β/Smad signaling pathways, including TβR-II, Smad2, and Smad4 (38). However, many tumor cells, without known mutations in these components, are resistant to growth inhibition by TGF-β. Understanding the mechanism by which tumor cells selectively lose this growth-inhibitory response to TGF-β is therefore important for a better understanding of the oncogenic processes.

We have investigated this problem in prostate cancer cells and have shown that overexpression of AR can repress Smad3-mediated transcriptional activation of TGF-β target genes in a ligand-dependent manner (unpublished data). Therefore, it is possible that AR may mediate the silencing of TGF-β antiproliferative responses in prostate cancer cells. In addition, DHT-mediated activation of AR function can be enhanced by the TGF-β/Smad signaling pathway in the presence of 1 μM HF and 10 nM E2, and Smad3 can further promote the transactivation of LNCaP mtARt877a. These results therefore provide evidence that growth factors such as TGF-β/Smad3 might be able to contribute to the increased agonist activity of HF and E2 to wtAR and mtAR in prostate cancer cells. In conclusion, our findings link the negative growth signals (TGF-β/Smad3) to positive growth signals (androgen/AR) in prostate cancer. Whether this pathway provides any potential therapeutic targets to battle prostate cancer growth remains to be further studied.

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