Pregnane X Receptor as a Therapeutic Target to Inhibit Androgen Activity

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The androgen-androgen receptor signaling pathway plays an important role in the pathogenesis of prostate cancer. Accordingly, androgen deprivation has been the most effective endocrine therapy for hormone-dependent prostate cancer. Here, we report a novel pregnane X receptor (PXR)-mediated and metabolism-based mechanism to reduce androgenic tone. PXR is a nuclear receptor previously known as a xenobiotic receptor regulating the expression of drug metabolizing enzymes and transporters. We showed that genetic (using a PXR transgene) or pharmacological (using a PXR agonist) activation of PXR lowered androgenic activity and inhibited androgen-dependent prostate regeneration in castrated male mice that received daily injections of testosterone propionate by inducing the expression of cytochrome P450 (CYP)3As and hydroxysteroid sulfotransferase (SULT)2A1, which are enzymes important for the metabolic deactivation of androgens. In human prostate cancer cells, treatment with the PXR agonist rifampicin (RIF) inhibited androgen-dependent proliferation of LAPC-4 cells but had little effect on the growth of the androgen-independent isogenic LA99 cells. Down-regulation of PXR or SULT2A1 in LAPC-4 cells by short hairpin RNA or small interfering RNA abolished the RIF effect, indicating that the inhibitory effect of RIF on androgens was PXR and SULT2A1 dependent. In summary, we have uncovered a novel function of PXR in androgen homeostasis. PXR may represent a novel therapeutic target to lower androgen activity and may aid in the treatment and prevention of hormone-dependent prostate cancer. (Endocrinology 151: 5721–5729, 2010)

Prostate cancer is the most common malignancy diagnosed in American men and the second leading cause of male cancer mortality (1). In the 1940s, Charles Huggins found that metastatic prostate cancer responded to androgen deprivation therapy (ADT) (2), which has since become the mainstay of treatment for locally advanced and metastatic prostate cancer.

The ADT strategies in prostate cancer patients include orchietomy and the use of GnRH (also known as LHRH) agonists or antagonists, steroidal and nonsteroidal antiandrogens, and inhibitors for the 5α-reductase (3). Orchietomy is a simple surgical procedure, but it has fallen out of favor given its psychological impact and viable medical alternatives for androgen deprivation (4). GnRH agonist therapy is widely used as a medical and reversible castration. GnRH agonists induce a transient increase in plasma testosterone (T) levels during the first week of treatment, causing a “flare” reaction in prostate cancer patients (5). GnRH antagonists do not cause a T surge, but they could be associated with an enhanced risk of ana-

Abbreviations: ADT, Androgen deprivation therapy; AR, androgen receptor; BrdU, bromodeoxyuridine; BW, body weight; CYP, cytochrome P450; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; EIA, enzymatic immunoassay; FABP-VP-PXR, fatty acid binding protein-viral protein 16-PXR; FBS, fetal bovine serum; hPXR, human PXR; IMDM, Iscove’s modified Dulbecco’s medium; LP, lateral prostate; LXR, liver X receptor; 6β-OH-T, 6β-hydroxytestosterone; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PCN, pregnenolone-16α-carbonitrile; PSA, prostate-specific antigen; PXR, pregnane X receptor; PXR-/-, PXR null; PW, prostate weight; R1881, methyltrienolone; RIF, rifampicin; shRNA, short hairpin RNA; siRNA, small interfering RNA; SULT, sulfotransferase; SULT2A1, hydroxysteroid SULT; T, testosterone; TG, transgenic; TO1317, N-methyl-n-(4-(2,2,2-trifluoro-1-hydroxy-1-trifluoromethyl)ethyl)-benzenesulfonamide; TP, testosterone propionate; T-Sulf, T sulfate; VP, ventral prostate; WT, wild type.
Phytoestrogens have also been used to block the effects of androgens. It was reported that antiandro- gens may cross the blood-brain barrier and block the negative feedback of androgens at the hypothalamic-pituitary level, which will promote the release of LH into the circulation, leading to a subsequent increase in serum T level (7). It has been recognized that ADT has limitations in that it provides a very significant but transient effect because of relapse as castration resistant prostate cancer.

Another major pathway to inhibit androgen activity is hormone metabolism, which includes the sulfotransferase (SULT)-mediated sulfoconjugation and cytochrome P450 (CYP)-mediated hydroxylation. SULTs belong to a family of phase II drug metabolizing enzymes that catalyze the transfer of a sulfonyl group from the cosubstrate 3'-phosphoadenosine-5'-phosphosulfate to the acceptor substrates to form sulfate or sulfamate conjugates (8). Sulfonation plays an important role in steroid hormone deactivation, because sulfonated hormones often fail to bind to and activate their cognate receptors and thus lose their hormonal activities (8–10). Sulfoconjugation also converts lipophilic steroid hormones to amphiphiles, which promotes their excretion (11). The primary SULT isoform responsible for androgen sulfonation is believed to be the hydroxysteroid SULT (SULT2A1) (11). In humans, SULT2A1 is expressed in steroidogenic organs, androgen-dependent tissue (prostate), digestive tract, and liver. In rodents, SULT2A1A9 is predominantly expressed in the liver (12). Changes in hepatic SULT2A1 expression can result in alterations in androgen hormonal activity and responsiveness. Androgen sensitivity of the rat liver was inversely correlated with the hepatic expression of SULT2A1 (10). On the other hand, the expression of SULT2A1 has been shown to be down-regulated by androgens (13), which may represent a regulatory mechanism to maintain sufficient androgenic activities. In addition to SULT2A1, the CYP3A enzymes also contribute to androgen deprivation through their oxidative deactivation of T (14).

Pregnane X receptor (PXR) is a nuclear receptor that can be activated by many exogenous and endogenous ligands (15, 16). These include the mouse PXR-specific ligand pregnenolone-16α-carbonitrile (PCN) and the human PXR (hPXR)-specific ligand rifampicin (RIF). After ligand binding, PXR forms a heterodimer with the retinoid X receptor that binds to PXR response elements, located in the 5′-flanking regions of PXR target genes. PXR has been established as a master regulator that controls the expression of drug-metabolizing enzymes and transporters. The PXR target genes include CYP3A and SULT2A1 (15–17). The CYP3As regulated by PXR include CYP3A4 in humans and CYP3A11, CYP3A13, CYP3A16, and CYP3A25 in mice (18). Having known that SULT2A1 and CYP3As are PXR target genes, we hypothesize that PXR may play a role in androgen deprivation by activating SULT2A1 and CYP3As.

In this study, we showed that in an in vivo model of castration and androgen replacement, activation of PXR reduced circulating levels of androgens and inhibited prostate regeneration. Treatment with PXR agonists inhibited the growth of human prostate cancer cells in SULT2A1- and PXR-dependent manner. We propose that PXR-mediated SULT2A1 and CYP3A gene activation represents a novel mechanism to lower androgen activity.

Materials and Methods

Animal models, prostate regeneration experiment, and bromodeoxyuridine (Brdu) immunostaining

The creation of fatty acid binding protein-viral protein 16-PXR (FABP-VP-PXR) transgenic (TG) mice (19) and PXR null (PXR−/−) mice (20) has been previously described. FABP-VP-PXR TG mice and their wild-type littermates were backcrossed to the CD-1 background for at least seven generations. The PXR−/− mice are maintained in mixed background of C57BL/6J and 129/SvJ. For the prostate regeneration experiment, mice were surgically castrated at 8 wk of age. Ten days after castration, mice received daily ip injection of T propionate (TP) (5 mg/kg) for 10 d to allow the prostate to regenerate (21). Mice were ip injected with BrDU (60 mg/kg) 6 h before being killed. When necessary, wild-type mice received PCN (daily ip in corn oil at 20 mg/kg) and/or TO/1317 (daily gavage at 50 mg/kg) treatment beginning 3 d before the TP treatment and continued until the completion of the experiment. The urogenital complex was removed and the anterior prostate, ventral prostate (VP), lateral prostate, and dorsal prostate lobes were separated under a dissecting microscope and weighed. VP lobes were processed for paraffin sections and subjected to Brdu immunostaining. The remaining prostate lobes from each mouse were then pooled for RNA extraction and gene expression analysis. For Brdu immunostaining, tissues were fixed in 4% formaldehyde, embedded in paraffin, sectioned at 5 μm, and subjected to immunostaining with a rat monoclonal anti-BrDU antibody (catalog no. OBT0030) from Accurate (Westbury, NY) at 1:20 dilution using the Vectastain Elite ABC kit from Vector Laboratories (Burlingame, CA). Diaminobenzidine tetrahydrochloride was used as the chromogen, and sections were counterstained with Gill’s hematoxylin. The use of mice in this study has complied with all relevant federal guidelines and institutional policies.

Cell proliferation assay

The androgen-dependent prostate adenocarcinoma LAPC-4 cells and their androgen-independent isogenic subline LA99 cells were maintained in Iscove’s modified Dulbecco’s medium (IMDM) from Invitrogen (Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS). Cells were seeded onto 24-well cell culture plates at the density of 5 × 10⁴ per well. After 24 h of incubation, cells were replaced with phenol red-free IMDM supplemented with 10% charcoal/dextran-stripped FBS, in the ab-
sence or presence of T (10 nM) and/or RIF (10 μM). Cells were replaced with fresh medium daily. After 4 d of treatment, the medium was replaced with 250 μl MTT solution (1 mg/ml, in phenol red-free IMDM) per well, and cells were incubated in a cell culture incubator at 37 C for 2 h. The MTT crystals were solubilized in dimethylsulfoxide at room temperature for 2 h, and 150 μl from each well were subjected to optical density analysis using the Ultramark Microplate Imaging System from Bio-Rad (Hercules, CA) at a wavelength of 570 nm with the background subtraction at 630 nm.

Measurement of serum levels of T

The serum T levels were determined using a T enzymatic immunoassay (EIA) kit from Cayman Chemical (Ann Arbor, MI). According the manufacturer’s specification, this EIA assay is highly specific for T (100%), whereas its specificity for esterified T and T sulfate (T-Sulf) is 0.11 and 0.03%, respectively. To avoid the interference of serum on the assay, all serum samples were extracted with diethyl ether before the EIA assay, after the extraction protocol that accompanies the EIA kit.

Plasmid constructs and transient transfections

The expression vector for androgen receptor (AR) and the prostate-specific antigen (PSA)-Luc reporter gene were previously described (21). The full-length mouse CYP3A11 cDNA was cloned by RT-PCR using the following pair of primers: 5’-CGCGGTACCGAGGGAACGATGAGGAT-3’ and 5’-CGCGCTAGCTCATGCTCCAGTTATGACTGCAT-3’. The CYP3A11 cDNA was digested with Asp718 and Nhel and inserted into the same enzyme-digested pCMX expression vector. The identity of the cDNA was verified by DNA sequencing. HepG2 cells were transfected on 48-well plates using the polyethyleneimine polymer transfection agent as previously described (21). For each triplicate transfection, 0.6 g of pcDNA-AR, and 0.3 g of pcDNA-SULT2A1 were used. Transfected cells were then treated with vehicle or androgens in medium containing 10% charcoal/dextran-stripped FBS for 24 h before harvesting for luciferase and β-gal assays. The transfection efficiency was normalized against the β-gal activity.

Real-time RT-PCR analysis

Total RNA was isolated from tissues or cell cultures using the TRIZOL reagent from Invitrogen. RT was performed with the random hexamer primers and the SuperScript RT III enzyme from Invitrogen following the manufacturer’s instructions. SYBR Green-based real-time PCR was performed with the ABI 7300 Real-Time PCR System. Data were normalized against the control of cyclophilin signals. The sequences for PCR primers are listed in Supplemental Table 1, published on The Endocrine Society’s Journals Online web site at http://endo.endojournals.org.

PXR and SULT2A1 RNA interference experiment

Short hairpin RNA (shRNA) constructs against the hPXR in the retroviral pRS backbone were obtained from OriGene (Rockville, MD). SULT2A1 small interfering RNA (siRNA) was purchased from QIAGEN (Valencia, CA). The sequence of siSULT2A1 is 5’-CCCGAAGAACUGAAGCUAAA-3’ (corresponding to nucleotides 699–721). The shRNA and siRNA transfections were carried out using Lipofectamine 2000 from Invitrogen. The siRNAs were added to the final concentration of 20 nM in transfection. Cells plated on 24-well plates (8 × 10⁴ cells per well, plated the day before transfection) were transfected for 6 h before being replaced with medium containing 10% FBS and were maintained in this medium for 24 h before drug treatment.

Statistical analysis

Results are expressed as means ± sd. Statistical analysis was performed using the unpaired student’s t test for comparison between two groups. Linear regression was used to analyze the association between T level and prostate weight (PW). P values of less than 0.05 were considered statistically significant.

Results

Activation of PXR in mice inhibited androgen-dependent prostate regeneration

The mouse prostate is sensitive to androgen stimulation. We used FABP-VP-PXR TG mice and PXR agonist PCN-treated wild-type (WT) and PXR−/− mice to examine the effect of PXR on androgen-dependent prostate regeneration. FABP-VP-PXR TG mice bear the expression of VP-PXR in the liver and intestine under the control of the FABP promoter (19). Created by fusing the VP16 activation domain of the herpes simplex virus to the amino terminal of the full-length hPXR, VP-PXR activates PXR-responsive genes in a constitutive manner (19). WT or TG mice were castrated at 8 wk of age. Ten days after castration, when the prostates have degenerated (21), mice were treated with TP (5 mg/kg, ip) for 10 d to allow the prostate to regenerate. Six hours before being killed, mice were labeled with BrdU (60 mg/kg, ip). The urogenital complexes were removed, and the anterior prostate, VP, lateral prostate, and dorsal prostate lobes were dissected and weighed. We found that the average weights of individual prostate lobes or the total PW, when measured as ratios of PW to body weight (BW), were significantly lower in TG mice than in WT mice (Fig. 1A). The retarded prostate regeneration in TG mice was accompanied by a decrease in prostate epithelial proliferation as determined by BrdU labeling and immunostaining (Fig. 1B). The BrdU labeling index in the VP of TG mice was approximately 29% of the WT mice (Fig. 1C). PW was indistinguishable between WT and TG mice before castration (Fig. 1D) or after castration but before the administration of TP (Fig. 1E). These results suggested that in the prostate, the effect of the transgene was specific for the androgen replacement-driven prostate regeneration.

The inhibition of androgen-dependent prostate regeneration was also observed in WT mice treated with the mouse PXR agonist PCN. In this experiment, 8-wk-old WT male mice were castrated. Seven days after castration, mice were randomly divided into two groups, with one group receiving daily ip injections of PCN in corn oil at 20 mg/kg beginning 3 d before the TP treatment, and the
control group receiving the vehicle. The PCN treatment continued until the completion of the experiments. Beginning at 10 d after castration, all mice received daily ip injections of TP (5 mg/kg) for 10 d before being killed and analyzed for prostate regeneration. As shown in Fig. 1F, the prostate regeneration was inhibited in PCN-treated mice. In WT mice and before the administration of TP, PW was indistinguishable between the castrated/vehicle-treated and castrated/PCN-treated groups (Supplemental Fig. 1). The inhibitory effect of PCN on prostate regeneration was abolished in PXR−/− mice (Fig. 1G), suggesting that the inhibition was PXR-dependent.

**Activation of PXR lowered the circulating concentration of T in castrated mice receiving a pharmacological dose of T**

To understand the mechanism by which PXR inhibited the androgen-dependent prostate regeneration, we measured the levels of T in the serum of TG mice and PCN-treated WT mice that were injected daily with TP. As shown in Fig. 2, the average serum T concentrations in TP-treated castrated WT mice was approximately 7 ng/ml, similar to what has been reported (21, 22). In contrast, the serum concentrations of T decreased significantly in TP-treated TG mice (Fig. 2A) and TP- and PCN-treated WT mice (Fig. 2B) compared with their WT and vehicle-treated control groups, respectively. In the PCN-treated mice, we observed a positive correlation between the measured T levels in individual animals and their prostate sizes (Fig. 2C).

**Activation of PXR induced the expression of CYP3As and SULT2A1/2A9, hydroxy testosterone and T-Sulf failed to activate AR, and a forced expression of CYP3A11 or SULT2A1 was sufficient to deactivate androgens**

When the gene expression in the liver was analyzed, we found that TG mice (Fig. 3A) and PCN-treated WT mice (Fig. 3B) showed markedly increased mRNA expression of CYP3A11, CYP3A13, CYP3A16, CYP3A25, and SULT2A1/2A9. The expression of SULT2A1/2A9 and CYP3As in the mouse prostate was...
Treatment with PXR agonist inhibited androgen-dependent prostate cancer cell growth in a PXR-dependent manner

The inhibition of androgen-dependent prostate regeneration led us to determine whether the activation of PXR affects androgen-dependent growth of human prostate cancer cells. Prostate cancer cells, including the LAPC-4 cells, have been reported to express functional PXR (23). In this experiment, AR-positive and androgen-dependent LAPC-4 cells seeded in medium containing charcoal/dextran-stripped FBS were treated with the hPXR agonist RIF (20 μM), in the absence or presence of exogenously added T. As expected, treatment with T induced 2.6-fold increases in cell growth as determined by the MTT assay (Fig. 4A). Treatment with RIF significantly inhibited the androgen-dependent LAPC-4 cell proliferation (Fig. 4A). The RIF effect was androgen dependent, because RIF had little effect on LAPC-4 cell growth in the absence of androgens. RIF had little effect on the growth of androgen-independent isogenic LA99 cells, regardless of the androgen treatment (Fig. 4A). LA99 cells were created by maintaining LAPC-4 cells in androgen-deprived/reduced media containing charcoal/dextran-filtered FBS for 12 months. Compared with the parent LAPC-4 cells, LA99 cells have significantly reduced AR expression (Fig. 4B). We then used PXR knockdown to determine whether the inhibitory effect of RIF on LAPC-4 cell growth was PXR dependent. In the control-scrambled shRNA-transfected cells, treatment with RIF significantly reduced T-responsive proliferation of LAPC-4 cells (Fig. 4C, left panel). In PXR shRNA (23)-transfected cells, despite a somewhat reduced T-induced proliferation, this effect was no longer inhibited by RIF (Fig. 4C). The LAPC-4 and LA99 cell transfection efficiency was estimated to be 65–80% (data not shown). We cannot exclude the possibility that the data presented here may reflect the growth of a mixed population of transfected and untransfected cells. The down-regulation of PXR expression in shRNA-transfected cells was confirmed by real-time PCR (Fig. 4D).

Activation of PXR induced the expression of SULT2A1 in LAPC-4 cells, and the expression SULT2A1 was required for the growth inhibitory effect of PXR agonist

It has been shown that the expression of SULT2A1 was induced in primary human hepatocytes treated with RIF (24). Here, we showed that the expression of SULT2A1 was also induced by RIF in LAPC-4 cells (Fig. 5A). Interestingly, the expression of CYP3A4 was not induced in RIF-treated LAPC-4 cells (Fig. 5A). To determine whether SULT2A1 activity is required for the growth inhibitory effect of PXR agonist, we examined the effect of RIF on

nearly undetectable, consistent with our previously observation (21).

6β-Hydroxytestosterone (6β-OH-T) and T-Sulf are the primary T metabolites upon CYP3A4 and SULT2A1 metabolism, respectively. We used transient transfection to determine whether 6β-OH-T and T-Sulf are indeed hormonally inactive. In this experiment, HepG2 cells were transfected with the expression vector for AR, together with the PSA promoter reporter gene (PSA-Luc). Transfected cells were then treated with T, 6β-OH-T, or T-Sulf for 24 h before luciferase assay. As shown in Fig. 3C, treatment with T induced the reporter gene activities, whereas the activation of reporter gene was not observed in 6β-OH-T or T-Sulf-treated cells (Fig. 3C).

To determine whether a forced expression of CYP3A11 or SULT2A1 is sufficient to deactivate androgens, HepG2 cells were transfected with AR and PSA-Luc, together with expression vector for CYP3A11 and/or SULT2A1, before being treated with T, dihydrotestosterone (DHT), or methyltrienolone (R1881) for 24 h. R1881 is a synthetic androgen. As shown in Fig. 3D, forced expression of either CYP3A11 or SULT2A1 inhibited T- and DHT-induced reporter gene activation. Transfection of both enzymes did not produce an additive or synergistic effect. Interestingly, R1881 was sensitive to deactivation by SULT2A1 but not by CYP3A11 (Fig. 3D). It is possible that the modification at the C6 position of R1881 prevented the 6β-hydroxylation, leading to R1881 resistance to CYP3A11. Figure 3E depicts the chemical structures of T and R1881.
LAPC-4 cell proliferation after SULT2A1 knockdown by siRNA. As shown in Fig. 5B, knockdown of the endogenous SULT2A1 in LAPC-4 cells was efficient to abolish the growth inhibitory effect of RIF. The efficiency of SULT2A1 knockdown was confirmed by real-time PCR (Fig. 5C).

A combined treatment of PXR and LXR agonists was not additive or synergistic in inhibiting androgen-dependent prostate regeneration

We have previously reported in similar models that activation of liver X receptor (LXR) also enhanced androgen metabolism and suppressed androgen-dependent prostate regeneration (21). To determine whether activation of PXR and LXR had an additive or synergistic effect, we treated WT mice with both PCN and TO1317 (a synthetic LXR agonist) before being analyzed for androgen-dependent prostate regeneration. As shown in Fig. 6A, treatment with both PCN and TO1317 inhibited prostate regeneration to an extent similar to treatment with PCN alone (Fig. 1F) or TO1317 alone (21). To directly compare the effect of individual and combined treatment of PCN and TO1317, we calculated the relative PW/BW ratio and plotted the results of three independent experiments together. As shown in Fig. 6B, a combined treatment of PCN and TO1317 was not additive or synergistic in inhibiting androgen-dependent prostate regeneration.

Discussion

Androgens play a major role in normal prostate development and in the pathogenesis of prostate cancer (25). It is believed that most of the androgen actions are mediated through AR, whose activation stimulates a cascade of events that are required for the initiation and progression prostate cancer (26). As such, the most effective endocrine therapy for prostate cancer has been the androgen ablation (27). Other than castration and the use of antiandrogens, an important pathway to deactivate androgens is through the enzyme-mediated metabolic inactivation. In this study, we revealed a novel PXR-controlled and SULT2A1- and CYP3As-mediated pathway of lowering androgen activity. Activation of PXR by genetic or pharmacological means was sufficient to at least partially inhibit androgen-responsive prostate regeneration and prostate cancer cell proliferation. Moreover, the PXR agonist effect on androgen activities was abolished in PXR/H11002/H11002 mice and PXR knockdown prostate cancer cells. We propose that the PXR-SULT2A1 and PXR-CYP3As pathways represent a novel mechanism to lower androgen activity.

SULT2A1 has been implicated in androgen homeostasis and prostate cancer. The expression of SULT2A1 is known to be subject to androgen regula-
tion. Activation of AR suppressed SULT2A1 gene expression (13), and the level of SULT2A1 expression was lower in androgen-dependent prostate cancer cells (10).

In addition to sulfonating and deactivating androgens directly, SULT2A1 also plays a role in the sulfation of dehydroepiandrosterone (DHEA), an important precursor for T biosynthesis. It was reported that approximately 50% of total androgens in men are synthesized from DHEA in peripheral tissues (28). A decreased DHEA sulfation is potentially involved in an increased lifetime availability of free DHEA for androgen synthesis. Indeed, increased DHEA to DHEA-S ratio was seen in African Americans who carry the SULT2A1 variants that are associated with decreased expression and activity of SULT2A1 (29). These results suggest that decreased expression of SULT2A1 may have contributed to unchecked androgen stimulation and cancerous transformation. It is also conceivable that reactivation of SULT2A1 gene expression may represent a novel therapeutic strategy to inhibit androgen-dependent prostate cancer growth.

CYP3As, the human CYP3A4 and the mouse CYP3A11 in particular, are the most abundant CYPs in the liver. CYP3As are responsible for the hydroxylation and oxidation of diverse endogenous and exogenous chemicals, including prescription drugs, steroid hormones, fatty acids, and bile acids (30). CYP3A4 is expressed in the liver, gut, colon, prostate, and breast. It has been suggested that individual variation in CYP3A4 expression may play a role in prostatic carcinogenesis through the modulation of sex hormone metabolism (14). CYP3A4 has an important role in the oxidative deactivation of T by metabolizing T to 2α,6β-H9252-hydroxytestosterone, 6β-OH-T, and 15β-hydroxytestosterone, which have less or no hormonal activities. A mutation in CYP3A4 may lead to a reduced potential to oxidize T, rendering a greater bioavailability of the circulating T, or an increased potential for the local conversion of T to DHT (14).

FIG. 4. Treatment with PXR agonist inhibited androgen-dependent LAPC-4 prostate cancer cell growth in a PXR-dependent manner. A, left panel, Treatment with PXR agonist RIF inhibited T-induced LAPC-4 cell proliferation as measured by MTT assay. Cells were maintained in medium supplemented with 10% charcoal/dextran-stripped FBS in the presence of vehicle, T (1 nm), RIF (20 μM), or both during the 4-d treatment period. Right panel, Neither T nor RIF had effect on the growth of LA99 cells under the same culture condition. All groups were normalized to the LAPC-4/vehicle group, which was arbitrarily set at 1. B, LA99 cells had decreased mRNA expression of AR compared with the parent LAPC-4 cells, as determined by real-time PCR. C, Down-regulation of PXR by shRNA abolished the growth inhibitory effect of RIF on LAPC-4 cells. The treatment conditions after shRNA transfection were identical to those described in A. All groups were normalized to the scrambled shRNA/vehicle group, which was arbitrarily set at 1. D, The efficiency of PXR knockdown was confirmed by real-time PCR. *, P < 0.05; **, P < 0.01.

FIG. 5. Activation of PXR induced the expression of SULT2A1 in LAPC-4 cells, and the expression SULT2A1 was required for the growth inhibitory effect of PXR agonist. A, Effect of RIF on the mRNA expression of SULT2A1 and CYP3A4 in LAPC-4 cells. Cells were treated with vehicle (dimethylsulfoxide) or Rif (20 μM) for 24 h before RNA extraction and real-time PCR. B, Knockdown of SULT2A1 by siRNA abolished the growth inhibitory effect of RIF on LAPC-4 cells. The treatment conditions after siRNA transfection were identical to those described in Fig. 4A. C, The efficiency of SULT2A1 knockdown was confirmed by real-time PCR. *, P < 0.05; **, P < 0.01. NS, Statistically not significant (P > 0.05).
SULT2A1 exerted its androgen inhibitory effect by activating the androgen-stimulated cell growth. Transactivation of either receptor suppressed androgen-dependent prostate regeneration in castrated mice, which was associated with decreased circulating levels of active androgens presumably derived mainly from androgen-dependent prostate regeneration. Results are presented as relative PW/BW ratio with the averages of each vehicle control groups arbitrarily set at 1. The numbers of mice in each group are labeled, which represent the total numbers of mice used from two to three independent experiments. *, P < 0.05; **, P < 0.01.

In addition to PXR, the nuclear receptor LXR has also been reported to play a role in androgen deprivation (21). Among the similarities of these two models, activation of either PXR or LXR inhibited the androgen-dependent prostate regeneration in castrated mice, which was associated with decreased circulating levels of active androgens presumably derived mainly from the administered TP. In cultured androgen-dependent prostate cancer cells, activation of either receptor suppressed the androgen-stimulated cell growth. Transactivation of SULT2A1 appeared to be a shared mechanism by which PXR and LXR promote androgen deprivation. There were also several notable differences between the PXR and LXR models. Compared with PXR, who activated both SULT2A1 and CYP3A, LXR exerted its androgen inhibitory effect by activating SULT2A1 but not CYP3A (21). Activation of LXR actually suppressed instead of activated CYP3A gene expression (31). Activation of LXR also inhibited the expression of steroid sulfatase in the prostate, which may have helped to prevent the local conversion of sulfonated androgens back to active metabolites. In contrast, PXR had little effect on the expression of steroid sulfatase (data not shown). Interestingly, a combined treatment of PXR and LXR agonists was not additive or synergistic in inhibiting androgen-dependent prostate regeneration (Fig. 6). In cell cultures, forced expression of both SULT2A1 and CYP3A11 was not more effective than forced expression of individual enzymes (Fig. 3D), suggesting that either sulfation or hydroxylation was sufficient to deactivate androgens. The hierarchy of PXR and LXR in regulating androgen homeostasis in vivo remains to be determined. Nevertheless, the shared function of these two nuclear receptors in androgen deprivation may represent a fail-safe mechanism to ensure the maintenance of a proper androgenic activity.

We have not observed overt reproductive effects in PXR TG mice despite the significant effect of PXR on androgen-dependent prostate regeneration in castrated mice. Although this could reflect a preferential first pass metabolism of ip administered TP, it is also possible that the enhanced T deactivation had not yet led to an obvious endocrine defect sufficient to trigger reproductive phenotypes, perhaps due to compensation by the hypothalamus-pituitary-testis axis.

In summary, we have revealed a novel function of PXR in lowering androgen activity in castrated male mice that received pharmacological doses of T and in cultured human prostate cancer cells, although the effect was insignificant with respect to reproductive physiology in intact mice. Our results suggest that activation of SULT2A1 and CYP3As may function in concert to mediate PXR-dependent suppression of androgen activity. Based on these data, PXR and its target androgen-metabolizing enzymes may represent therapeutic target for hormone-dependent prostate cancer. We recognize that there are potential challenges in using PXR manipulation as a strategy to lower androgen activity. These include the potential of drug-drug interaction associated with PXR activation. Another potential liability is the PXR-induced sensitization to oxidative stress, although this effect, previously reported for the liver (19), is yet to be confirmed for the prostate. It is also unclear whether activation of PXR is effective in reducing intratumor androgen levels.

Acknowledgments

We thank Dr. Zhou Wang for the R1881 compound and Dr. Daotai Nie for his assistance in the PXR shRNA experiment.

Address all correspondence and requests for reprints to: Dr. Wen Xie, Center for Pharmacogenetics, University of Pittsburgh, Pittsburgh, Pennsylvania 15261. E-mail: wex6@pitt.edu. This work was supported by in part by National Institutes of Health Grants ES014626 and DK076962. Z.O. is sup-
ported by a scholarship from the China Scholarship Council (no. 2008638059).

Disclosure Summary: The authors have nothing to disclose.

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