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Cross-talk between androgen receptor and pregnane and xenobiotic receptor reveals existence of a novel modulatory action of anti-androgenic drugs

Subodh Kumar, Bharti Jaiswal, Sanjay Kumar, Seema Negi, Rakesh K. Tyagi *

Special Centre for Molecular Medicine, Jawaharlal Nehru University, New Delhi 110067, India

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ABSTRACT

The androgen receptor (AR) is a member of nuclear receptor superfamily (NRs) and plays a critical role in prostate cancer development and progression. Therefore, anti-androgens that repress AR activity remain a critical mainstay for prostate cancer therapy. However, molecular mechanisms by which anti-androgens exert their therapeutic effects are not clearly elucidated and hence are a major area of scientific pursuit. Here, we demonstrate that another member of NRs, pregnane and xenobiotic receptor (PXR), not only acts as a molecular sensor of anti-androgens but also influences the outcome of therapeutic regimen with anti-androgenic drugs via a novel AR–PXR cross-talk. Using 'gain- and loss-of-function' studies, we identified a distinct role of PXR as a potent repressor of AR-regulated transcription. Our study implicates PXR as a key determinant of anti-androgen action since down-regulation of PXR diminishes the potency of the anti-androgenic drugs and enhances transcriptional actions of androgens. In addition, our subcellular localization studies revealed that ligand-activated AR induces nuclear localization of PXR and the two receptors colocalize at discrete sites in nucleus and mitotic chromatin. Finally, we report a distinct antagonist-induced interaction between AR and PXR defining a hitherto unidentified mode of action of AR antagonist. In this perspective, the study may help in designing and development of novel AR antagonists offering improved avenues in prostate cancer therapy.

1. Introduction

Androgen receptor (AR), a member of nuclear receptor superfamily (NRs), mediates the effects of male steroid hormones (androgens) and contributes to a wide variety of physiological and patho-physiological conditions. Aberrant androgen signaling is known to lead to several endocrinopathies like prostate cancer, male infertility etc. [1,2]. Presently, anti-androgens which block the AR signaling are the standard first line of therapy for initial stages of prostate cancer. Unfortunately, the benefit of this therapy is only transitory and all patients eventually relapse with tumor cells whose growth escapes the androgen deprivation [3]. The details of molecular mechanisms involved in anti-androgen-mediated AR repression are not yet well understood. For the development of more efficacious therapies for prostate cancer, it is therefore imperative to elucidate how anti-androgens work to understand their circumstantial failure. Extensive investigations have revealed that complex networks of sequentially exchanged coactivator/corepressor complexes are required to generate precise and complex program of AR-mediated gene expression. These coregulator complexes possess sensing activities required for interpretation of multiple endogenous and exogenous signals. Binding of androgen to AR induces the recruitment of coactivators like SRC-1, GRIP-1 and p300 thus triggering the activation of AR-regulated genes. In contrast, binding of antagonist induces AR to form a complex with corepressors, such as NCoR and SMRT, that leading to repression of AR-regulated genes. Thus, these modulatory factors influence the amplitude of transcriptional activity of AR in response to its ligands and furthermore, also exhibit ligand-selective differences [4,5].

Mammalian cells contain a variety of receptors that can bind and respond to environmental xenobiotics. These primarily include the NRs members such as pregnane and xenobiotic receptor (PXR), Constitutive Androstane Receptor (CAR) and Aryl hydrocarbon Receptor (AhR) [6,7]. PXR is distinct among all nuclear receptors as it is highly promiscuous having broad ligand specificity. It is activated by structurally diverse array of exogenous and endogenous compounds like rifampicin, troglitazone, cholesterol etc. leading to the induction of drug metabolizing enzymes such as CYP3A4 [8,9]. While the role of PXR is clearly established as a 'xenosensor' as well as in the regulation of drug metabolism related genes, its presence and functions in other tissues remain ambiguous. Recent reports of PXR expression in cancerous tissues i.e. breast, endometrial, prostate and ovarian cancers imply that PXR has distinct functions in these tissues thereby suggesting its role in patho-physiological situations [10–14]. Preliminary reports

* Corresponding author. Tel.: +91 11 2674 1544; fax: +91 11 2674 1781.
E-mail addresses: rktyagi@yahoo.com, rkt2300@mail.jnu.ac.in (R.K. Tyagi).

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indicate that few endocrine disruptors are capable of inducing CYP3A4, through activation of PXR [15,16]. Although anti-androgens and endocrine disruptors are believed to repress AR activity, a number of these are reported to activate PXR suggesting the existence of an alternative pathway via which these chemicals may impart anti-androgen action. Therefore, the existence of a modulatory cross-talk between these two signaling pathways is apparent.

There are evidences indicating that AR-mediated transcriptional activity may be modulated by other nuclear receptors and transcription factors. For instance, signaling molecules like Daxx, orphan nuclear receptor AhR, TR-4 and TR-2 have been shown to be negative modulator of AR transactivation [17–20]. Rather than inhibiting AR activity, polypeptide growth factors have been shown to stimulate AR-mediated transcription [21]. Along similar lines, PXR also negatively regulate other signaling pathways like HNF4α and NF-κB [22,23]. These observations suggest that molecular cross-talks play an important role in nuclear-receptor-mediated gene regulation.

The present study demonstrates a novel role of PXR in the regulation of AR-dependent genes. Our results identify PXR as a repressor of AR, repressing AR-mediated transcriptional activity and also suggest its role as a 'molecular sensor' of anti-androgens and endocrine disruptors as these compounds induce PXR transcriptional activity. Interestingly, a unique antagonist-promoted interaction between AR and PXR is also revealed in the present study suggesting PXR's implicit role in antagonist-mediated transcriptional repression of AR signaling. Finally, we provide evidence to prove that down-regulation of PXR diminishes the potency of anti-androgenic prostate cancer drugs and enhances the transcriptional actions of androgens. These observations of inhibitory cross-talk between AR and PXR signaling pathway may be utilized in the development of novel AR antagonists that offer improved avenues in therapeutic regimen of prostate cancer.

2. Materials and methods

2.1. Reagents and biochemicals

All plastic wares for mammalian cell culture were purchased from Corning Costar Corp. (Corning, NY, USA). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), dextran-charcoal-stripped serum and other mammalian cell culture reagents were procured from Hyclone (Logan, UT, USA). Anti-androgens and PXR activators used in this study were from Sigma Chemicals Co. (St.Louis, MO, USA). Methoxychlor was purchased from Crescent Chem. (USA). Casodex was a kind gift from Astra Zeneca UK. Antibodies against full-length human AR and PXR were developed in our laboratory and described earlier [24,25]. RevertAid first strand cDNA synthesis kit were products of Fermentas (USA). Primers for PCR were purchased from Sigma Chemicals Co. (Bangalore, India). All the reagents for PCR amplification were procured from New England Biolabs (Beverly, MA, USA). Luciferase assay kit was obtained from Promega (Madison, WI, USA). All other chemicals (unless otherwise mentioned) were obtained from Sigma Chemicals Co. (St.Louis, MO, USA) or local commercial sources supplying molecular biology grade reagents.

2.2. Plasmids

Mammalian expression constructs for wild-type human androgen receptor, pSG5-hAR and GFP-tagged human AR (GFP-AR) were described earlier [24]. Promoter-reporter constructs, ARE3-tk-Luc containing three copy numbers of probasin promoter ARE sequence and designated as ARE-tk-Luc and PSA-Luc, containing the luciferase gene under the control of a 5.7-kb-promoter fragment of the human PSA gene were kindly provided by Dr. O.A. Janne (University of Helsinki, Finland) and Dr. Charles Bieberich (University of Maryland, Baltimore, MD, USA), respectively. The constructs pSG5-hPXR and XREM-CYP3A4-Luc (XREM-Luc) have been described earlier [26]. Expression construct of human GR and its promoter-reporter GRE-Luc were kind gifts from Dr. Sagar Sengupta (National Institute of Immunology, India) and Dr. H. Tanaka (University of Tokyo, Japan), respectively. The mammalian two-hybrid constructs VP16-GR and FR-LUC were generous gifts from Dr. S. Stoney Simons, Jr. (NIDDK, NIH, Batheseda, USA). VP16-AR was kindly provided by Dr. Donald McDonnell (Duke University Medical centre, Durham, USA). The constructs Gal4DBD-PXR, SUPER vector encoding PXR-siRNA pSUPER-siPXR and pSUPER vector were a gift from Dr. Jongsook Kim Kemper (University of Illinois, Urbana-Champaign, Illinois, USA). Mice PXR was subcloned from YFP-mPXR (a kind gift from Dr. Masahiko Negishi, NIEHS, NIH, Research Triangle Park, NC, USA) into pDsRed-Express-C1 vector (BD Biosciences Clontech, Palo Alto, USA) using XhoI and EcoRI restriction sites and designated as RFP-mPXR.

2.3. Maintenance of cell lines

Kidney cell lines from African green monkey COS-1 and CV-1, human liver cell line HepG2, and human prostate cancer cell lines DU-145 and LNCaP were obtained from National Centre for Cell Science repository (Pune, India). All cells were grown in DMEM supplemented with 10% FBS, 100 μg/ml penicillin and 100 μg/ml streptomycin while LNCaP cells were grown in Ham's F12K with 15% FBS supplemented with 0.1 nM DHT and antibiotics. HepAR (HepG2 stably transfected with human AR) and HepXR (HepG2 cells stably transfected with human PXR) cells developed in our laboratory were maintained in the same DMEM complete medium containing 200 μg/ml of G418 [24,25]. The cultures were maintained in a humidified incubator at 5% CO_2 and 95% air atmosphere at 37 °C.

2.4. Transient transfections and reporter gene assays

Cells were cultured in 12-well plates in DMEM with 10% FCS (Hyclone, Logan, UT, USA) to ~80% confluency. Cells were transiently transfected by mixing the indicated amounts of plasmid DNA with 2 μl/well of Escort III/IV (Sigma, St. Louis, MO, USA) according to manufacturer's protocol. After 12 h of transfection period, the culture medium was replaced with 1 ml of DMEM, 10% charcoal-dextran-stripped fetal bovine serum (CS-FCS) (Hyclone, Logan, UT, USA) and treated with ligands as per experimental requirement. After another 24 or 48 h (for the pSUPER-siPXR experiments) the cells were rinsed twice with PBS and subsequently lysed in reporter lysis buffer and measured for luciferase activity using a luciferase reporter assay system (Promega, Madison WI, USA).

2.5. RNA extraction and reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA from cells and tissues was isolated using TRI reagent™ (Sigma, St. Louis, MO, USA) according to the manufacturer's instructions. The concentration of total RNA was determined by reading the O.D. at 260 nm. 2 μg of total RNA was used as template for reverse transcription by using the RevertAid first strand cDNA synthesis kit (Fermentas, USA). PCR amplification was done with Taq polymerase for 35 cycles (for PXR) or 30 cycles (for PSA). Each PCR product was separated on 1.5% agarose gel and visualized by ethidium bromide staining. In

Table 1
Sequences of primers used in the present study for RT-PCR.

Primer	Sequences (forward and reverse)	Annealing temperature (°C)	Product size (bp)
PXR (Human)	5′-GAG GAC ACA GAG TCT GTT CCT GGA AAG CCC-3′ 5′-GCT CCC TGA TCA TCA TCC GCT GCT CC-3′	60	410
PXR (Mice)	5′-CTG AGG TTC AAC ACG ATG TTC GAC AC-3′ 5′-GCC ATG ATC TTC AGG AAC AGG AAC CT-3′	60	329
PSA (Human)	5′-GGC AGG TGC TTG TGG CCT CTC-3′ 5′-CAC CCG AGC AGG TGC TTT TGC-3′	65	522
GAPDH (Human)	5′-CGA GAT CCC TCC AAA ATC AAG-3′ 5′-GTC TTC TGG GTG GCA GTG AT-3′	60	323
GAPDH (Mice)	5′-CTC ATG ACC ACA GTC CAT GC-3′ 5′-CAC ATT GGG GGT AGG AAC AC-3′	60	201

parallel, ubiquitously expressed GAPDH cDNA was amplified as control. The primers used in cDNA amplification that generated an amplified fragment size of 410 bp for human PXR, 329 bp for mouse PXR, 522 bp for human PSA,323 bp for human GAPDH and 201 bp for mouse GAPDH are tabulated in Table 1.

2.6. Western blot analysis

Isolation of proteins from cultured mammalian cells and mice tissues were performed with TRI reagent™ (Sigma, St. Louis, MO, USA) using manufacturer's protocol. For Western blotting each of the extracted fractions was dissolved and denatured in SDS sample buffer by heating at 95 °C for 5 min. Equal amount of proteins were resolved by 10% SDS-PAGE. Proteins were transferred to polyvinyldifluoridine (PVDF) membrane using semi-dry transfer system (Amersham Biosciences). Following transfer, PVDF membrane was stained with Ponceau S to confirm the efficiency and uniformity of protein transfer. The membrane was blocked with 5% quick blocker (Genotech, USA) dissolved in TBS for 2 h at room temperature and then incubated with PXR or AR antiserum at a dilution of 1:1000 and 1:5000, respectively, for overnight at 4 °C. The PVDF membrane was then washed three times with TBST (TBS with 0.1% Tween-20) and incubated for 1 h with 1:10,000 dilution of horseradish peroxidase-conjugated anti-rabbit secondary antibody (Sigma, St. Louis, MO, USA). The bound antibody complexes were detected using the enhanced chemiluminescence (ECL) system.

2.7. Analysis of intracellular localization using fluorescence microscopy

COS-1 cells were seeded in 35 mm cell culture plates in DMEM supplemented with 10% FBS and were cultured overnight. The cells were transfected for 8 h with 0.5 μg of each plasmid using Escort III. Following the transfection period, the cells were supplemented with fresh DMEM containing 5% charcoal-stripped FBS and treated with or 10^{-8} M 5α-dihydrotestosterone (DHT) or 10 μM rifampicin or 10 μM pregnenolone 16α-carbonitrile (PCN) and incubated further for 24 h. To facilitate the visualization of the nucleus, nuclear stain Hoechst (1 μg/ml) was used. The fluorescent cells were viewed through a Nikon upright fluorescence microscope model 80i equipped with water immersion objectives and connected to cooled CCD digital camera (model Evolution VF, Media Cybernetics Inc., Maryland (USA)). Images were captured and analyzed with Image ProPlus version 5.0 software (Media Cybernetics Inc., Maryland (USA)). The images were processed using standard image processing techniques. Localization was considered nuclear (N) when fluorescence was exclusively or predominantly stronger in nucleus. When the fluorescence was uniformly distributed between the nuclear and cytoplasmic compartment it was designated (N = C). When receptor was present exclusively in the cytoplasm than nucleus it was designated (C). For localization score at least 100 fluorescent cells were counted in each experiment.

2.8. Mammalian two-hybrid assay

CV-1 cells were seeded in 12-well plates and transiently co-transfected (with Escort IV) with the indicated amounts of plasmids VP16-AR (encoding VP16 transactivation domain fusion protein with AR) together with a Gal4-DBD-PXR (encoding Gal4 DNA binding domain fusion protein with PXR) along with promoter-reporter plasmid FR-Luc (in 1:1:5 ratio), containing the luciferase reporter gene with upstream five tandem repeats of the 17-bp GAL4-binding element, as a read out for interaction. Stimulation in luciferase activity indicated the interaction between the two receptors. Luciferase activities were assayed as described above.

2.9. Co-immunoprecipitation assay

In vivo co-immunoprecipitation assays were performed with COS-1 cells co-transfected with 500 ng of pSG5-AR and pSG5-PXR plasmids. The cells were treated with 10 nM DHT or 1 μM anti-androgens/PXR activators for 24 h post-transfection and harvested with lysis buffer (20 mM Tris–HCl, 300 mM NaCl, 0.5% NP40, protease inhibitors, 100 μM PMSF). Whole cell lysate was incubated with 1 μl of PXR polyclonal antibody or preimmune serum overnight at 4 °C. Each portion was further incubated for additional 2 h after addition of 20 μl of Protein A agarose bead slurry (Sigma, St. Louis, MO, USA). Agarose beads were washed three times with lysis buffer at 4 °C and bound proteins were dissolved in Laemmli's SDS-PAGE sample buffer. Proteins were separated by 10% SDS-PAGE. Proteins from gel were transferred onto PVDF membrane (MDI, India) and subjected to Western blot with anti-AR antibodies. Signals were then detected with ECL kit. Quantification of band intensities was done by Alpha Ease FC software (Alpha Imager HP, version 5.01, Alpha Innotech Corporation San Leandro, CA, USA).

2.10. Statistical analysis

Most of the experiments were done at least three times in duplicates and values represent the means ±SD of three separate experiments. Statistical analysis was done by two way Student's *t*-test and the figures labeled with asterisks (*) signify values that differed significantly from the control experiment with *p*-value less than 0.05 ($p < 0.05$ in Student's *t*-test).

3. Results

3.1. Anti-androgens exert inverse response on transcriptional activity of AR and PXR

PXR is a highly promiscuous nuclear receptor which can be activated by structurally diverse groups of xenobiotic and endobiotic compounds. In this context, we attempted to explore whether the established anti-androgens activate PXR or not? To investigate this, promoter-reporter-based luciferase assays were

performed in CV-1 cell line. Cells were transiently transfected with XREM-Luc reporter gene and PXR expression plasmid and incubated with a series of anti-androgens. All tested anti-androgens were able to activate PXR transcriptional activity significantly albeit to differing extent (Fig. 1A). To corroborate these findings, we also examined the effect of these anti-androgenic compounds and potent PXR activator rifampicin on AR transcriptional activity. Interestingly, along with the conventional anti-androgens, the PXR activator rifampicin also significantly repressed the agonist-mediated AR activity (Fig. 1B). Our results suggest that most anti-androgens and endocrine disruptors, which are believed to repress AR activity, are capable of activating PXR indicating the possible role of PXR in anti-androgen-mediated functions.

3.2. PXR is endogenously expressed in prostate tissue and prostate cancer cell lines

While PXR expression is well documented in the liver and intestine, the expression of PXR in other tissues remains intriguing and controversial. With respect to present study an obvious question arises whether PXR is expressed in the prostate tissue, which is a key site of AR expression and function. RT-PCR and Western blot assays were performed to investigate the expression of PXR in AR positive prostate cancer cell line LNCaP and AR negative cell line DU145, as well as in mice prostate tissue. In RT-PCR analysis, PXR mRNA expression was detected in both the cell lines and also in mice prostate tissue. We observed an amplified PCR product of expected size (410 bp) for human PXR mRNA in human prostate cancer cell lines LNCaP and DU145 by using a set of primers specific to human PXR. Similarly, using mice PXR primers, anticipated band of 323 bp was also observed in mice prostate tissue (Fig. 2A). To examine the expression of PXR at protein level, Western blot was performed using polyclonal antibody against PXR. In Western blot analysis, the major PXR band of relative molecular mass 50 kDa was detected in all the cell lines and also in mice prostate tissue (Fig. 2B). Taken together, our results exhibited the endogenous expression of PXR in prostate cancer cell lines and prostate tissue at both mRNA and protein levels.

3.3. Agonist-mediated activity of AR is negatively regulated by PXR

To investigate the possibility of existence of functional cross-talk between AR and xenobiotic receptor PXR, transient transfections were performed in CV-1 cells. For this purpose, CV-1 cells were co-transfected with ARE-tk-Luc promoter-reporter and AR expression plasmid either in the presence or in the absence of PXR expression plasmid. Androgen (DHT) leads to significant increase in AR-mediated transactivation of ARE-tk-luciferase reporter activity. Interestingly, co-transfection of PXR, significantly repressed DHT-mediated AR transcriptional activity in presence of PXR activator rifampicin. However, moderate level of PXR-

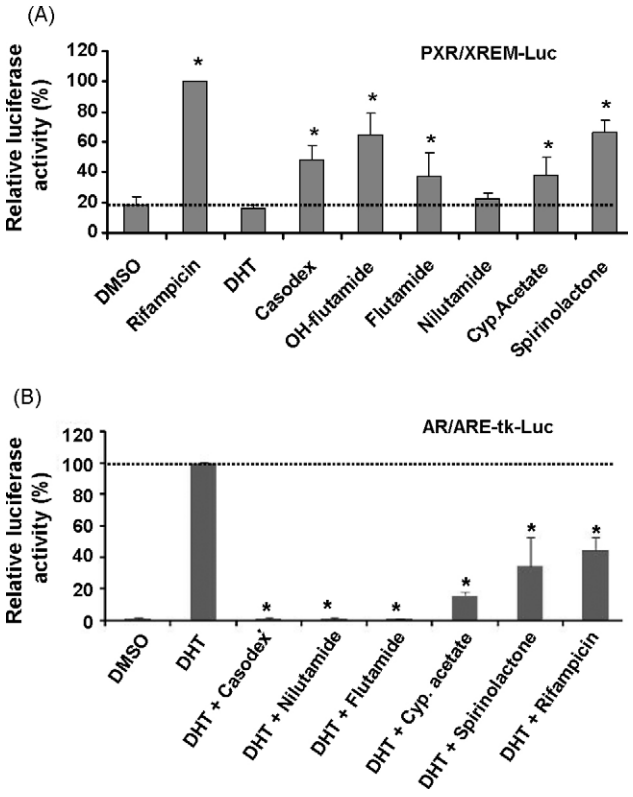

Fig. 1. Effect of anti-androgens/PXR activators on PXR and DHT-mediated AR transcriptional activity. (A) The effect of anti-androgens on PXR transcriptional activity. CV-1 cells were transiently co-transfected with XREM-Luc reporter gene along with PXR expression plasmids and incubated for 24 h with 10 μM of indicated anti-androgens and 10^{-8} M of DHT. DMSO (vehicle) was added to control cells. After incubation period, cells were harvested for luciferase assay. The relative values for reporter luciferase are plotted using PXR response to rifampicin as 100%. (B) The effect of anti-androgens/PXR activators on DHT-mediated transcriptional activity of AR. CV-1 cells were transiently co-transfected with ARE-tk-Luc along with AR expression plasmid and incubated for 24 h in the absence (control) or in the presence 10^{-9} M of DHT and 10^{-6} M of different anti-androgens and 10 μM rifampicin in combination with DHT. DMSO (vehicle) was used as control. After 24 h of incubation, cells were harvested for luciferase assay. The relative values for reporter luciferase are plotted using AR response to DHT as 100%. The values represent the means ±SD of three separate experiments. Asterisks (*) signify values that differed significantly from the luciferase value of vehicle treated (A) and DHT alone treated (B) cells ($p < 0.05$ in Student's *t*-test).

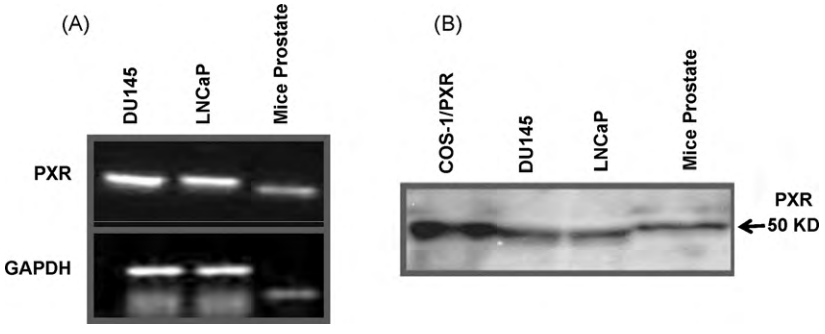

Fig. 2. Analysis for endogenous expression of PXR in prostate cancer cell lines and mice prostate tissue. Total RNA and protein from DU145, LNCaP cells and prostate tissue were simultaneously prepared with TRI reagent and used for RT-PCR and Western blot analysis, respectively. For mice prostate, Swiss albino male mice were sacrificed and prostate tissue was isolated and total RNA and protein was isolated from the same tissue sample. (A) RT-PCR analysis of mRNA expression for PXR in DU145, LNCaP and mice prostate tissue. GAPDH was used as an internal control for mRNA expression level. (B) Western blot analysis of PXR protein in prostate cancer cell lines and mice prostate tissue. Equal amount of proteins were electrophoresed through 10% SDS-PAGE, blotted on to PVDF membrane and probed with anti-PXR antibodies (1:1000 dilutions). COS-1 cells transiently transfected with human PXR expression plasmid were used as positive control for PXR expression.

mediated suppression of AR activity was also observed in absence of rifampicin which is not surprising since, PXR is also activated by many endogenous compounds [7–9]. These results suggest that ligand bound PXR significantly inhibits AR-mediated transcriptional activity (Fig. 3A).

We also performed similar experiments using a promoter reporter that contains the human Prostate Specific Antigen (PSA) promoter. PSA is a well-studied androgen responsive gene expressed in prostate cells and is a prognostic marker of prostate cancer. Similar to ARE reporter, exogenously co-transfected PXR

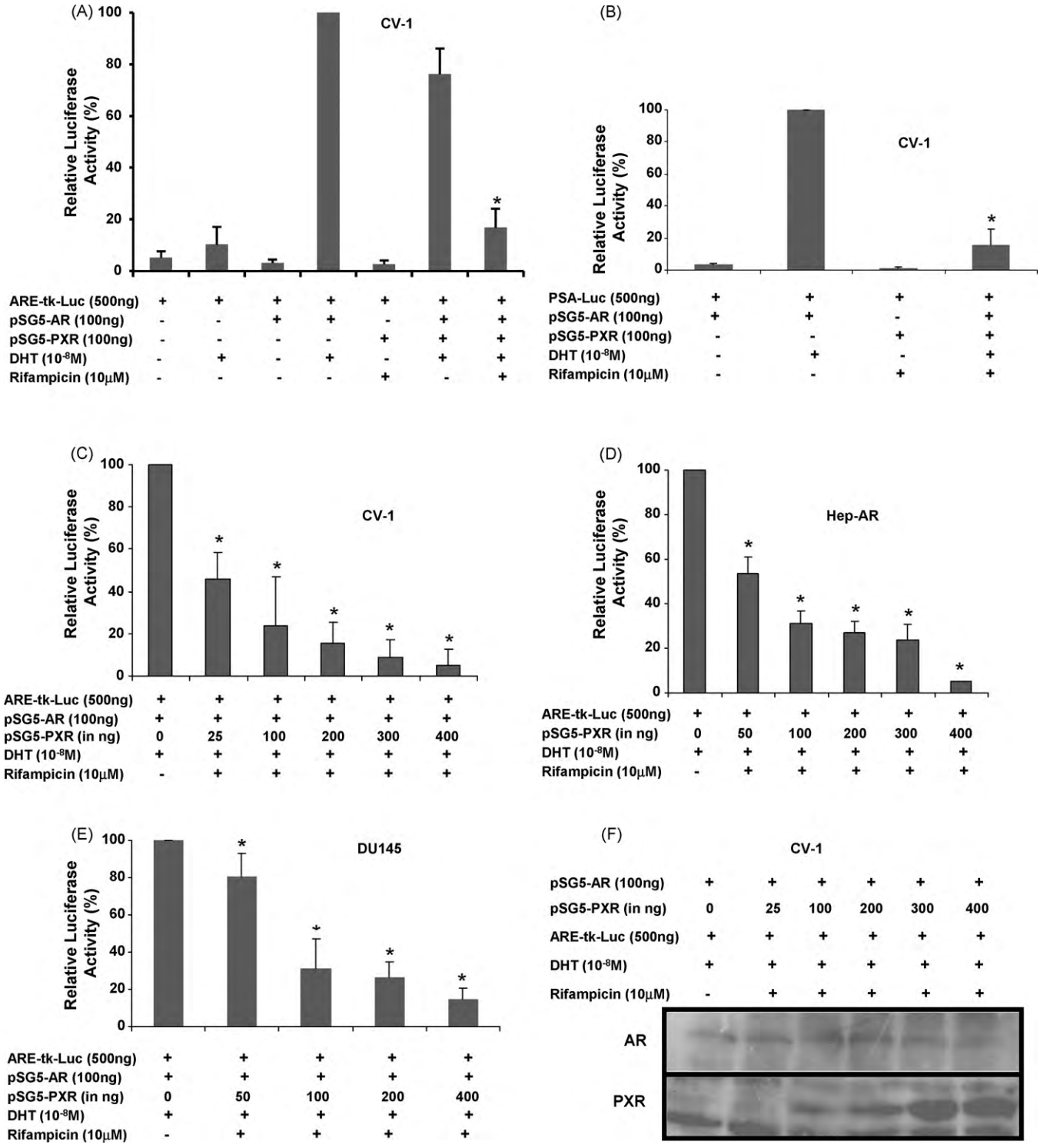

Fig. 3. Repression of AR-mediated transcriptional activity by PXR. ARE-tk-Luc (A) and PSA-Luc (B) and different combinations of PXR and AR expression plasmids were co-transfected in CV-1 cells, according to scheme shown in the figure. (C–E) The repression of AR-mediated transactivation in a dose-dependent manner by PXR in CV-1, Hep-AR and DU145 cells, respectively. CV-1 (C), Hep-AR (D) and DU145 cells (E) were co-transfected with promoter-reporter ARE-tk-Luc along with AR expression plasmid and increasing concentration of PXR expression plasmid according to the scheme shown in the figure. Following the transfection period, the cells were incubated for 24 h in the absence (DMSO as control) or in the presence 10^{-8} M of DHT and 10 μM of rifampicin alone or in combination with DHT. Following the period for treatments, the cell extracts were prepared and used for assaying transcriptional response as described under Section 2. The relative values for reporter luciferase are plotted using AR response to DHT as 100%. The values represent the means ±SD of three separate experiments. Asterisks (*) signify values that differed significantly from the luciferase value of DHT alone treated cells ($p < 0.05$ in Student's *t*-test). (F) testified no effect of PXR on AR protein expression/stability. Western blot analysis of AR and PXR was performed by the same lysates used for luciferase assays in CV-1 cells for (C).

significantly repressed DHT-mediated AR transcriptional activity on PSA promoter (Fig. 3B). These results demonstrate that PXR can competitively repress the AR-mediated transcriptional activity on both the PSA and the ARE promoters in CV-1 cells.

To determine whether PXR represses AR-mediated transactivation in a dose-dependent manner, increasing amounts of PXR expression plasmid were co-transfected in monkey kidney cell line CV-1, in HepAR cells (HepG2, human liver cell line stably expressing AR) and in human prostate cancer cell line DU145. Increasing amount of PXR expression plasmid exhibited progressive repression in DHT-mediated AR transcriptional activity suggesting the inhibitory effect of PXR on AR transactivation in a dose-dependent manner. In view of the fact that we observed the repressive effect of PXR in CV-1 (Fig. 3C), HepAR (Fig. 3D) and DU145 (Fig. 3E) cell types the results suggested that PXR-mediated repression of AR is not a cell type specific event.

To exclude the possibility that the dose-dependent repression of AR activity by a non-specific transcription squelching mechanism because of strong promoters present on co-transfected expression plasmids that is added in a dose-response fashion, we examined the effect of increasing dose of potent and specific PXR activator, rifampicin on AR activity in Hep-AR cells that stably express AR and also have endogenous of PXR. Interestingly, increasing concentrations of rifampicin also showed progressive repression in DHT-mediated AR transcriptional activity (Supplementary Fig. 1). The observations support the view that inhibitory effect of PXR on AR activity in a dose-dependent manner is a specific event and is not due to the phenomenon of transcriptional squelching.

To investigate if PXR executes its action by modulating levels of AR protein, we performed Western blot analysis of AR and PXR using the same lysates that were used for luciferase assays in CV-1 cells. No significant effect on AR expression levels was observed with increasing level of PXR implying that reduction in AR-mediated activity is neither due to the inhibition of AR expression nor because of the potential artifactual effects linked to exogenous transfection of PXR (Fig. 3F).

To examine if PXR also modulates transactivation of other closely related nuclear receptors, we tested the effect of PXR on promoter-reporter construct of glucocorticoid receptor (GR). There was no effect of PXR on ligand-induced transactivation of the GRα indicating that PXR is not a general inhibitor of nuclear receptors rather it appeared to be specific for AR (Supplementary Fig. 2). These experiments indicate that PXR may be selective in its functional interactions with some nuclear receptors and of the receptors tested it only interfered with AR transactivation. However, as we have studied the effect of PXR on only two members of nuclear receptor superfamily, the data obtained do not exclude the possibility that PXR can modulate the activity of some other members of the superfamily.

3.4. Silencing of endogenous PXR level enhances the agonist-mediated activity and diminishes the AR repressive potency of anti-androgens

To corroborate the observed repression of AR transcriptional activity by PXR, we attempted 'loss-of-function' approach to

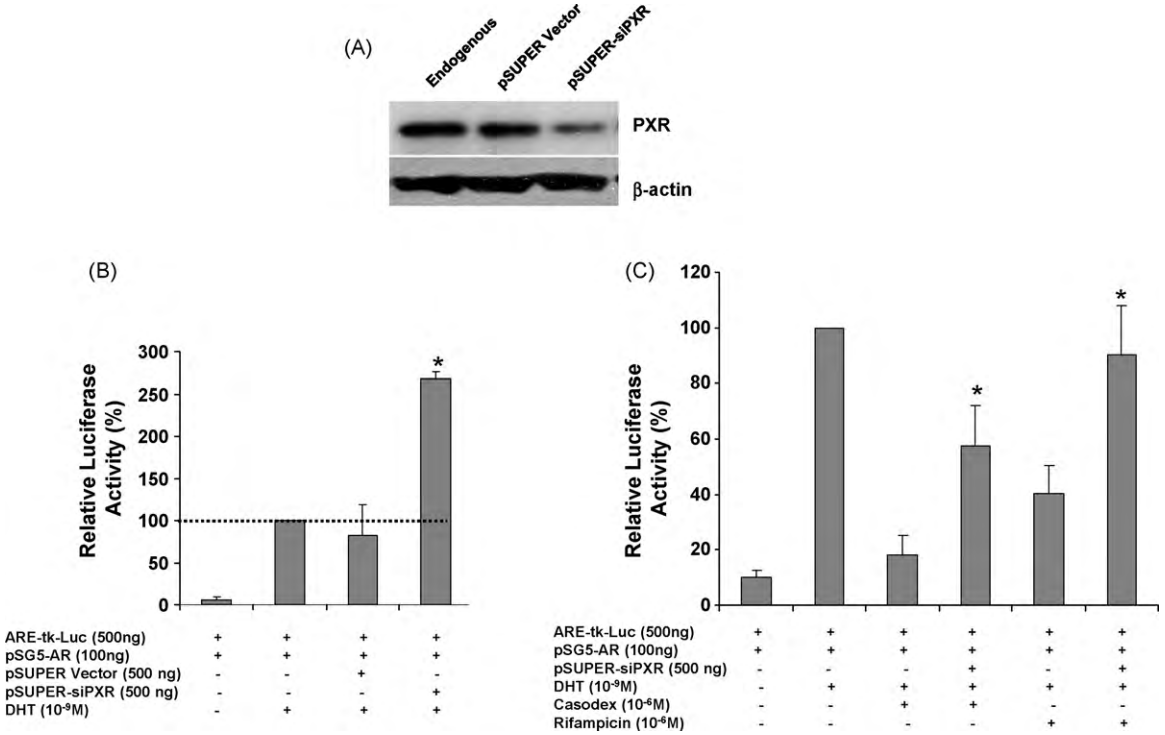

Fig. 4. Effect of PXR knockdown on DHT- and antagonist-mediated AR transactivation function. (A) The down-regulation of PXR protein by siRNA-PXR in HepG2 cells. HepG2 cells were transfected with siRNA-PXR expression plasmid (pSUPER-siRNA-PXR) or pSUPER vector (control). After 48 h of expression, cell lysate was prepared and PXR protein level was ensured by Western blot analysis using anti-PXR antibodies (1:1000 dilutions). Lane 1 indicates untransfected HepG2 cell lysate, Lane 2 indicates pSUPER transfected HepG2 cell lysate and Lane 3 indicates pSUPER-siPXR-PXR transfected HepG2 cell lysate. β-actin was used as protein loading control. (B) The augmentation of DHT-mediated AR transcriptional activity by down-regulation of endogenous PXR. Promoter-reporter ARE-tk-Luc, AR expression plasmid and different combinations of siRNA-PXR plasmid or control vector (pSUPER) were co-transfected in HepG2 cells, according to the scheme shown in the figure. Following transfection period, the cells were treated with 10^{-8} M of DHT for 48 h and then harvested for luciferase assay. (C) The diminishing of antagonist-mediated repression of AR activity by silencing of PXR. ARE-tk-Luc, AR expression plasmid and different combinations of siRNA-PXR plasmid were co-transfected in HepG2 cells, according to scheme shown in the figure. After transfection incubation, the cells were treated with 10^{-9} M of DHT alone or in combination with 10^{-6} M of antagonists, casodex or rifampicin for 48 h and then harvested for luciferase assay. The relative values for reporter luciferase are plotted using AR response to DHT as 100%. The values represent the means ±SD of three separate experiments. Asterisks (*) signify values that differed significantly from the luciferase value of pSUPER vector with DHT-treated cells for (B) and in absence of siRNA-PXR with DHT in combination with antagonist (casodex vs. casodex, rifampicin vs. rifampicin) treated cells for (C) ($p < 0.05$ in Student's t-test).

examine if down-regulation of endogenous PXR has some influence on AR activity. For this purpose, specific siRNA was used to investigate the role of endogenous PXR on AR-mediated gene expression. Endogenous PXR protein level was evaluated by Western blot analysis using PXR antibody. PXR protein level correlated with the result of our reporter gene assay as over-expression of PXR siRNA significantly reduced the PXR expression (Fig. 4A). Promoter-reporter assays with AR were performed in HepG2 cells either in the presence of siRNA construct targeting PXR (pSUPER-siPXR) or with control vector (pSUPER vector). AR transcriptional activity was significantly enhanced by expression of PXR siRNA but not with the control vector (Fig. 4B). Taken together these data indicated that endogenous PXR functions as a repressor of AR activity.

To determine if PXR has a role in antagonist-mediated AR-dependent transcriptional repression, we assessed the effect of reduced cellular level of PXR using siRNA, on the regulation of AR transcriptional activity in response to pure anti-androgens, casodex and a potent PXR activator, rifampicin. Both casodex and rifampicin significantly repressed the DHT-stimulated AR activity. Interestingly, down-regulation of PXR by expression of siPXR partially reversed both casodex- and rifampicin-mediated repression of AR transcriptional activity (Fig. 4C). Collectively, these data suggest that PXR contributes to antagonist-mediated repression of AR activity.

3.5. AR represses transcriptional activity of PXR

To determine whether the AR–PXR cross-talk is bidirectional in nature, we also assessed the effect of AR on PXR transcriptional activity. HepXR (HepG2 cells stably transfected with human PXR) cells were co-transfected with XREM-Luc promoter reporter along with the increasing concentration of AR expression plasmid. PXR activator rifampicin led to significant increase in PXR-mediated transactivation of the XREM-luciferase reporter activity. Interestingly, co-expression of AR led to dose-dependent decrease in rifampicin-mediated PXR transcriptional activity (Fig. 5). AR-mediated repression of PXR activity suggests the bidirectional nature of AR–PXR cross-talk as both the receptors repress each other's transcriptional activity.

3.6. Ligand-activated AR promotes nuclear translocation and mitotic chromatin association of PXR

Modulation of AR transcriptional activity by PXR prompted us to examine if the two receptors interact/colocalize in the cells. Studies with GFP-AR fusions have confirmed that unliganded AR resides mainly in the cytoplasm and on addition of DHT it translocates into the nucleus with formation of 'nuclear foci' [27–29]. Recently, it has been shown that DHT-activated GFP-AR associates with the mitotic chromatin whereas unliganded AR does not [24]. Furthermore, studies to determine the subcellular localization of PXR have provided conflicting results. Some reports suggest that irrespective of ligand binding, PXR is an exclusively nuclear protein and remains constitutively associated with mitotic chromatin [26]. However, other reports suggest that in unliganded condition PXR remains in cytoplasm and upon activation by its agonists it translocates to the nucleus [30]. The discrepancies in these findings may be attributed to the differences in the type of PXR employed (human vs. mouse) or nature of the tagged constructs used in the studies. These reports indicate that subcellular trafficking is an important regulatory process for the transcription function of PXR. To study the subcellular dynamics of PXR we subcloned mice PXR in DsRed-express vector and designated as RFP-mPXR. Interestingly, unliganded RFP-mPXR showed predominately cytoplasmic localization and upon addition of its agonists like PCN it translocated to the nucleus with formation of distinct 'nuclear foci' (Fig. 6A). In addition, RFP-mPXR also showed agonist-mediated mitotic chromatin association while the unliganded PXR showed homogenous distribution during mitosis (Fig. 6A). We also assessed the transcriptional function of RFP-mPXR and confirmed it to be transcriptionaly active (not shown).

In subsequent studies, RFP-mPXR was employed to study the possible molecular interaction/colocalization of AR and PXR. COS-1 cells were co-transfected with equimolar amount (500 ng) of GFP-AR and RFP-mPXR. In absence of AR agonist DHT both AR and PXR showed predominantly cytoplasmic localization (Fig. 6B). Furthermore, in unliganded condition both AR and PXR were excluded from mitotic chromatin (Fig. 6B). Upon treatment of cells with AR agonist DHT, AR translocated to the nucleus with formation of 'nuclear foci'. Surprisingly, ligand-activated AR also induced the

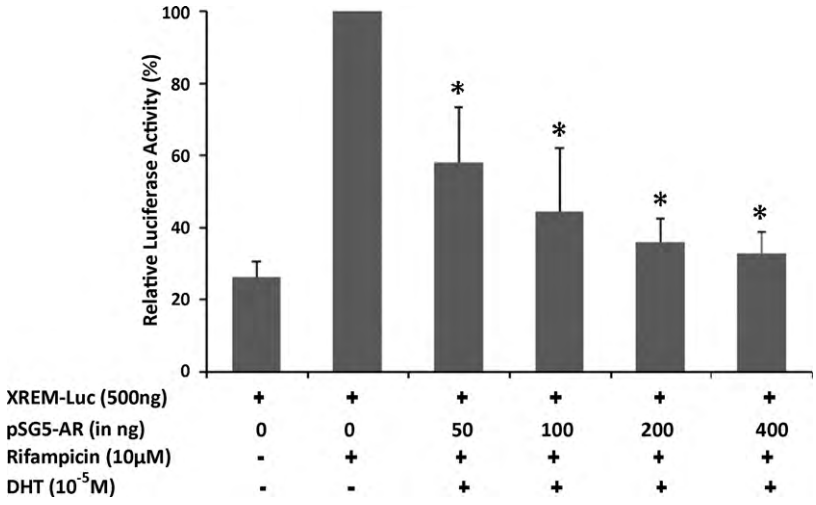

Fig. 5. AR represses PXR-mediated transactivation. XREM-Luc promoter reporter along with increasing concentration of AR expression plasmid were co-transfected in HepXR cells according to scheme shown in figure. After transfection and expression period, the cells were treated with rifampicin (10 μM) and DHT (10^{-8} M) for 24 h (as indicated in scheme) and then harvested for luciferase assay. Relative fold activity was calculated in comparison to rifampicin-induced luciferase activity which is taken as 100%. Values are average of three independent experiments performed in duplicates. Asterisks (*) signify values that differed significantly from the luciferase value of rifampicin alone treated cells ($p < 0.05$ in Student's t-test).

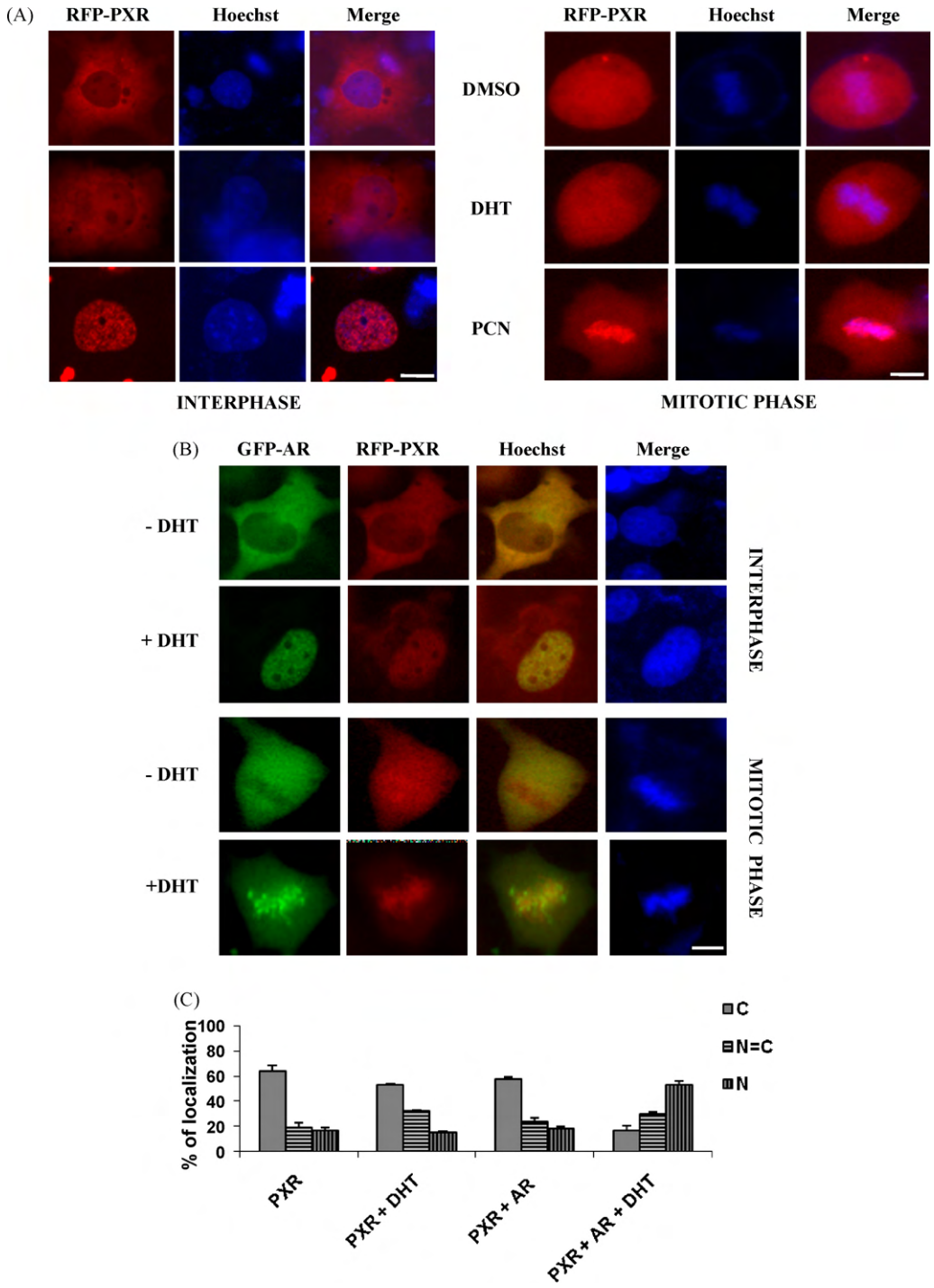

Fig. 6. Effect of DHT-activated AR on subcellular localization of PXR during interphase and mitosis. (A) The subcellular localization of PXR in interphase and on mitotic chromatin. COS-1 cells were transfected with 500 ng of RFP-mPXR. Following transfection and receptor expression period, cells were treated with either 10^{-8} M DHT or 10 μM PCN for 24 h. (B) The effect of DHT-activated AR on PXR subcellular localization. COS-1 cells were co-transfected with equimolar amount (500 ng) of GFP-AR and RFP-mPXR. Following transfection and receptor expression period, cells were treated with 10^{-8} M DHT for 2 h. Subcellular localization of RFP-mPXR and GFP-AR during interphase and mitosis was monitored and recorded by fluorescence microscope. In all the cases, cells were incubated with Hoechst (1 μg/ml) to visualize the corresponding nuclei or mitotic chromosomes. (C) The graphical representation of subcellular localization of RFP-mPXR. For localization score, at least 100 fluorescent cells were counted in each experiment as described in Section 2. The values represent the means ±SD of three separate experiments. Asterisks (*) signify values that differed significantly from the percentage value of DHT-treated RFP-mPXR alone cells ($p < 0.05$ in Student's *t*-test). Scale bar, 5 μm.

translocation of PXR to the nuclear compartment with the formation of nuclear foci and both AR and PXR were observed to be colocalized in the nucleus within similar nuclear foci (Fig. 6B). In addition, ligand-activated AR also recruited PXR onto mitotic chromatin (Fig. 6B). To confirm that DHT-activated AR is essential for nuclear translocation and mitotic chromatin association of PXR, we tested the effect of DHT alone on dynamic localization of RFP-mPXR. DHT alone could neither induce the nuclear translocation nor mitotic chromatin association of PXR (Fig. 6A middle, left and right panels). This indicated that nuclear translocation of PXR is AR mediated. Additionally, to validate our results statistically, we estimated the percentage of cells displaying a distinct localization pattern of RFP-mPXR alone or with co-expression of AR, both in the absence or presence of DHT. Scoring of cells demonstrated nuclear

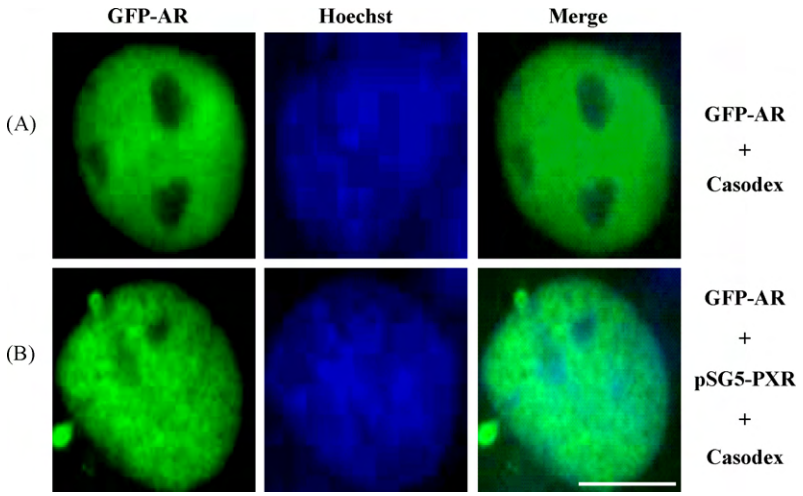

Fig. 7. Redistribution of antagonist-bound AR by co-expression of PXR. COS-1 cells were transiently transfected with 500 ng of GFP-AR alone (A) or in combination with 500 ng of pSG5-PXR (B). Following the transfection, the cells were treated with 10^{-6} M casodex for 24 h. After 24 h of expression, subnuclear pattern of GFP-AR were monitored and recorded by fluorescence microscope. In all the cases, cells were incubated with Hoechst (1 μg/ml) to visualize the corresponding nuclei. Scale bar, 5 μm.

localization of RFP-mPXR in a significant number of DHT-treated cells which co-express the two receptors as compared to cells where RFP-mPXR was expressed alone or co-expressed in the absence of DHT (Fig. 6C). Our data suggest that liganded AR shuttles PXR to the nucleus as well as to the mitotic chromatin. This nuclear and chromatin association of AR and PXR may modulate the transcriptional activity of AR in distinct manner.

3.7. PXR redistributes antagonist-bound AR into 'nuclear foci'

It has been well established that agonist as well as pure antagonist-bound AR translocates to the nucleus. However, there is striking difference in subnuclear distribution pattern in agonist and pure antagonist-bound AR. Agonist-bound AR is concentrated transiently in subnuclear compartments that has the appearance of fine granules termed as 'nuclear foci', while AR-bound to an antagonist (pure anti-androgen), such as casodex or nilutamide, is distributed more uniformly in different pattern throughout the nucleus [27–29]. The transient accumulation of androgen-bound AR in nuclear foci might be mediated by interactions between AR and its coregulatory proteins that are specific to the conformation of the AR [28,29]. Involvement of PXR in antagonist-mediated repression of AR led us to hypothesize that it may also alter the subcellular distribution of antagonist-bound AR. The subcellular distribution of GFP-AR was investigated in casodex-treated cells. In agreement with previous observations, casodex-treated GFP-AR is found to be localized in the nucleus showing a predominantly diffuse distribution. Interestingly, co-expression of wild-type human PXR leads to the alteration of nuclear pattern of casodex-bound GFP-AR from diffuse to punctuate, nuclear foci pattern (Fig. 7A and B). This PXR-dependent subnuclear redistribution of antagonist-bound AR suggests that PXR may interact with antagonist-bound AR and support the hypothesis that PXR may be involved in antagonist-mediated repression of AR activity.

3.8. PXR interacts physically with AR which is augmented by AR antagonists/PXR activators

To determine whether the transcription repression reflects a direct interaction between PXR and AR, mammalian two-hybrid experiments were carried out using VP16 transactivation domain fusion protein with AR together with a Gal4-DBD-PXR and a Gal4-luciferase reporter gene (FR-Luc) as a read out for interaction (Fig. 8A). The Gal4-PXR fusion protein alone had negligible transcriptional activity when transfected with FR-Luc promoter-reporter construct. When both fusion proteins were co-expressed, interaction of Gal4-PXR with VP16-AR was apparent from the two-hybrid signal. However, in the presence of AR agonist DHT, we observed less interaction between AR and PXR than in unliganded condition (Fig. 8B). In our negative control set co-transfection with VP16-GR there was no stimulation in Gal4-PXR activity.

One of the most pertinent questions arising from the present study was how different AR antagonists/PXR activators would affect AR–PXR interaction in the intact cell? The mammalian two-hybrid experiments were performed in presence of a series of AR antagonists. A ligand-independent interaction between PXR and AR is detected. Surprisingly, PXR–AR interaction was markedly enhanced by AR antagonists and PXR activators i.e. rifampicin, methoxychlor, troglitazone, spirinolactone and casodex. In our negative control experiment, co-transfection assay with VP16-GR did not show any stimulation in Gal4-PXR activity (Fig. 8C). These observations suggest that there is a direct interaction between PXR and AR. Enhanced interaction of PXR with AR in presence of AR antagonists suggests that PXR might contribute to transcriptional regulation of antagonist-bound AR and regulate the magnitude of the hormone response.

Co-immunoprecipitaion experiments further supported the ligand-mediated modulation of AR–PXR interaction between the two receptors. COS-1 cells were co-transfected with AR and PXR expression plasmids and treated for 24 h either with DHT or AR antagonists/PXR activators i.e. casodex, rifampicin and methoxychlor. Immunoprecipitations performed using anti-PXR antibody, followed by Western blot analysis for AR revealed that AR efficiently interacted with PXR in both ligand-dependent and -independent manner. In comparison to input bands of AR and PXR showing similar levels of expression in all the lysates, there were clear differences among co-immunoprecipated AR band intensities in response to various ligands (Fig. 8D). Similar to the results obtained from mammalian two-hybrid assays, the interaction between PXR and AR was higher in the presence of PXR activators/AR antagonist rifampicin, methoxychlor and casodex while this interaction was slightly low in the presence of AR agonist DHT (Fig. 8D and E). Ligand-dependent modulation of AR–PXR interaction strongly suggests that recruitment of PXR by AR is enhanced by AR antagonists and PXR activators.

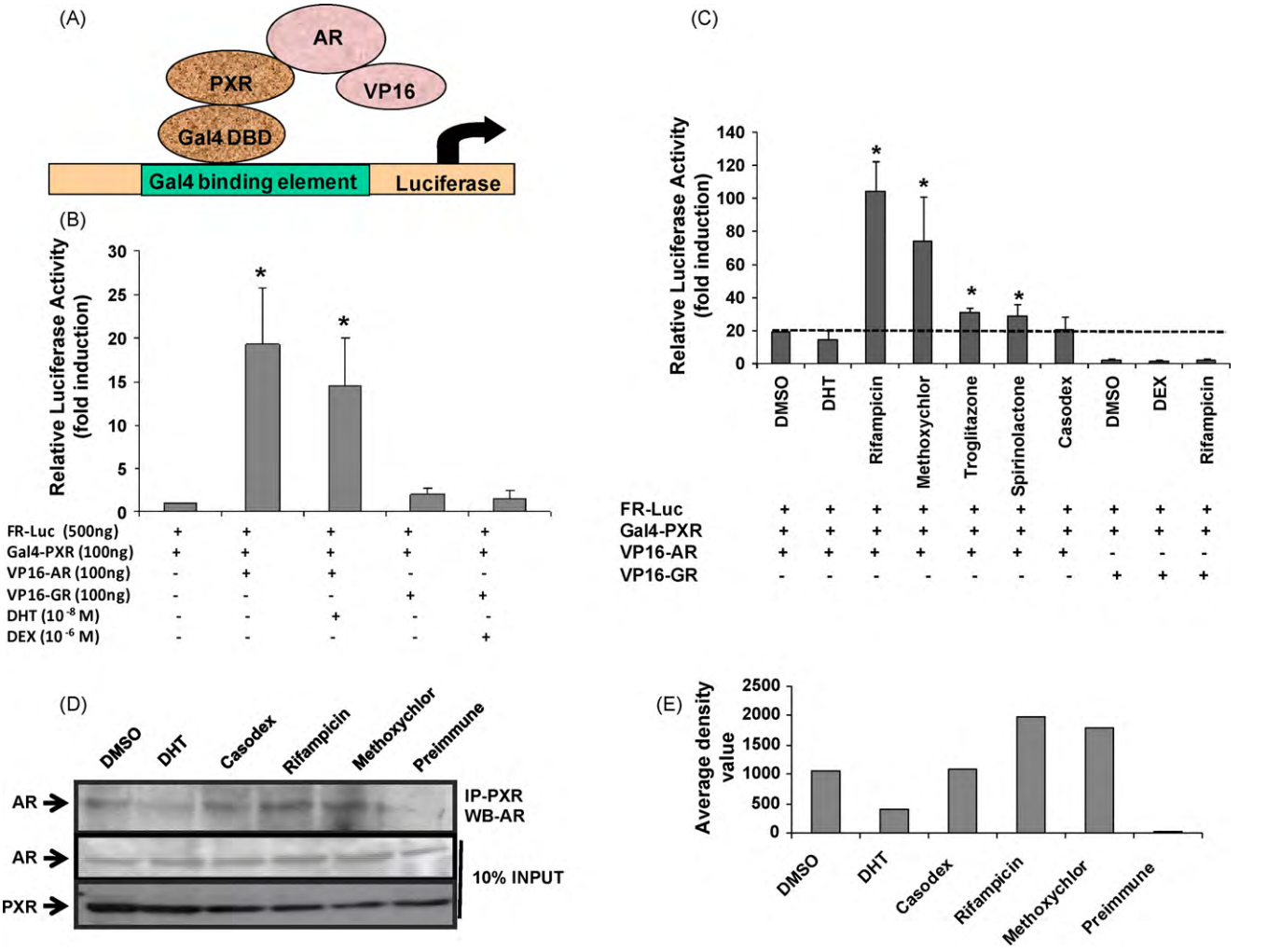

Fig. 8. PXR physically interacts with AR and the interaction is augmented by AR antagonists/PXR activators. (A) A simplified working scheme for the mammalian two-hybrid assays. (B) The interaction between AR and PXR. Full-length AR fused to VP16 (VP16-AR) and full-length PXR fused to Gal4-DNA binding domain (Gal4-PXR) were co-transfected in CV-1 cells along with Gal4 promoter-reporter FR-Luc. Following transfection, the cells were treated with indicated ligands for 24 h as specified and then harvested for luciferase assay. VP16-GR was used as negative control. (C) The augmentation of AR–PXR interaction by AR antagonists/PXR activators. VP16-AR and Gal4-PXR were co-transfected in CV-1 cells along with FR-Luc. After transfection incubation, the cells were treated with indicated ligands for 24 h as indicated in the experimental scheme and then harvested for luciferase assay. VP16-GR was used as a negative control. All values represent mean of ±SD of three separate experiments. Asterisks (*) signify values that differed significantly from the luciferase value of Gal4-PXR alone transfected cells for (B) and DMSO alone treated cells for (C) ($p < 0.05$ in Student's t-test). (D) AR–PXR physical interaction by co-imunoprecipitation. COS-1 cells were co-transfected with equimolar amount (500 ng) of wild-type PXR and AR expression plasmids. Cells were treated with indicated ligands as in the scheme for 24 h and harvested for immunoprecipitation with anti-PXR antibody as described under Section 2. Immunoprecipitated complex were detected by Western blotting using anti-AR antibody. IP, Immunoprecipitation; WB, Western blot. For input 10% of lysate was directly dissolved in SDS-PAGE sample buffer and Western blot was performed with anti-AR, anti-PXR and anti-β-actin antibodies. (E) The quantitation of band intensities of co-immunoprecipitated AR from (D). Band intensities that appeared in response to the indicated ligands were quantitated by using Alpha imager software.

3.9. PXR activators suppress the DHT-induced PSA expression in prostate cancer cells

Prostate specific-antigen (PSA) is a clinically significant androgen-stimulated gene that is used to monitor the response to treatment, the prognosis, and the progression of prostate cancer. To establish the functional significance of PXR in modulating AR-mediated gene expression, we examined the effect of PXR activators rifampicin and methoxychlor on expression of endogenous PSA in LNCaP cells. PSA expression level was measured by semi-quantitive RT-PCR and DHT-mediated induction in level of PSA transcript was evident in the cells treated with 1 nM DHT. Interestingly, PXR activators, rifampicin and methoxychlor lead to significant decrease in DHT-induced PSA expression (Fig. 9). However, there was no significant effect of these compounds on PSA level in absence of androgen, ensuring that PXR activators have no non-specific effect on the PSA gene and only inhibit AR-

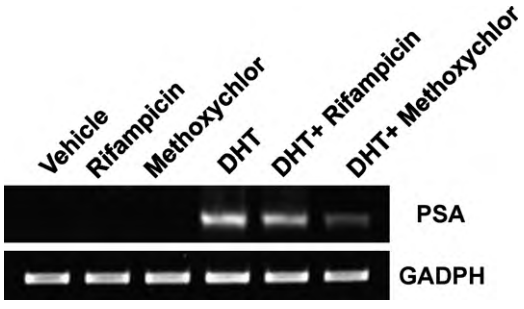

Fig. 9. Down-regulation of DHT-stimulated PSA transcript by PXR activators. LNCaP cells were grown in six well culture plates in steroid stripped serum and treated with 10^{-9} M of DHT, 10 μM of PXR activators, rifampicin and methoxychlor alone or in combination as per the scheme for 24 h and then harvested for RT-PCR analysis of mRNA expression for PSA gene. GAPDH was used as an internal control for mRNA expression level.

mediated gene expression. These results further substantiate the notion that PXR functions as a negative AR coregulator and down-regulates AR-mediated gene expression.

4. Discussion

Anti-androgens are a mainstay of prostate cancer therapy. However, mechanisms by which they exert their effects are not well understood. In an endeavour to delay or reverse the failure of such therapies, it is important to understand how anti-androgens work. Competition with ligand for binding undoubtedly contributes but cannot entirely account for anti-androgen action since the relative binding affinities of AR for anti-androgens are relatively lower than those for androgens [31]. Although the recruitment of corepressors like NCoR-1 and SMRT are well documented with certain anti-androgens; however, the possibilities of involvement of some other undefined factors cannot be ruled out [4,5]. In this scenario, it is reasonable to speculate that anti-androgens may also target a number of additional proteins and signaling pathways that contribute to transcriptional repression of AR. Our preliminary cell-based screening of known anti-androgens for PXR activity revealed that nearly all the AR antagonists behave as PXR agonist. Furthermore, potent PXR activator rifampicin efficiently repressed the AR activity. The discrete response of these compounds via AR and PXR strengthens our hypothesis of possible cross-talk between AR and PXR. Our results also complement previous studies which have demonstrated that PXR activators like troglitazone, methoxychlor, RU-486, DDT compounds; bisphenol A, T0901317 etc. also possess anti-androgenic activity [32–36]. Opposing effects of these compounds on AR and PXR suggest an inverse functional relationship between the two receptors. Furthermore, co-existence of AR and PXR in prostate fortifies our assumption of potential convergence of action of these two nuclear receptors.

To test our hypothesis, we did a series of 'gain-of-function' and 'loss-of-function' experiments to determine the effect of PXR on AR activity and documented that PXR behaves as a potent repressor of AR signaling. PXR specifically repressed AR activity in dose-dependent manner with two distinct AR reporter genes in different cell lines. In contrast, down-regulation of PXR by using siRNA augmented AR transcriptional activity. Finally, experimental evidences suggest that down-regulation of PXR diminishes the potency of the anti-androgenic prostate cancer drugs and enhances the transcriptional actions of androgens. These results advocate the involvement of PXR in antagonist-mediated repression of AR transcriptional activity. We also confirmed the bidirectional nature of AR–PXR cross-talk as AR was also able to repress PXR-mediated transcription. The repressive activity of PXR is not surprising since earlier reports have demonstrated that PXR can also negatively regulate other signaling pathways like NF-κB and HNF4α [22,23]. Furthermore, a previous report confirmed the presence of a cryptic repressor domain within PXR by Gal4 fusion experiments [37]. It will be interesting to know if the same domain is also involved in AR repression.

So, how does PXR repress AR-mediated gene transcription? Transcriptional cross-talk often involves protein–protein interactions between the participating factors. In an attempt to explore the molecular mechanisms involved in PXR-mediated repression of AR signaling, we enquired whether AR transcriptional repression reflects a direct interaction between PXR and AR. In our subcellular localization studies, we demonstrated that AR and PXR colocalize in cellular discrete sites in both interphase and mitotic cells suggesting the existence of close proximity of these two receptors inside the cell. In unliganded condition, AR colocalizes with PXR in cytoplasmic compartment and both AR and PXR exclude from mitotic chromatin. Interestingly, DHT-induced AR also translocates cytoplasmic PXR into the nucleus and colocalizes with PXR in interphase as well as in mitotic chromatin. Moreover, PXR alters the subnuclear distribution of antagonist-bound AR from homogenous to speckled pattern. Furthermore, using mammalian two-hybrid assay and co-immunoprecipitation, we confirmed that PXR physically associates with AR in mammalian cells. Interestingly, we also found PXR activators/AR antagonists markedly enhance the interaction between AR and PXR. This ligand-selective modulation of AR–PXR interaction by AR agonists and antagonists fortifies our view that PXR plays an important role in androgen-antagonist-mediated AR-dependent gene repression. Altogether, these results suggest that PXR activators/AR antagonists induce the recruitment of PXR to AR suggesting a novel mechanism of action of AR antagonists. Our findings suggest that the phenomenon of AR–PXR cross-talk is more prominent in patients undergoing anti-androgen therapy that leads to the enhanced AR repressive activity of these drugs. Nevertheless, our data also indicate that alteration in AR–PXR cross-talk may explain a possible mechanism by which prostate cancer acquires resistance to anti-androgen therapy.

Since transcription repression is a complex and multistep process and the chromatin environment that surrounds the genes plays vital role in AR-mediated gene expression, it will be interesting to know how AR–PXR interaction leads to the formation of a repressive complex. Involvement of some corepressors and chromatin remodeling factors could provide an explanation for the occurrence of this phenomenon. In this context, there are reports indicating that PXR interacts with corepressors like NCoR-1 and SMRT to execute its repressive actions [37]. Additionally, a recent observation suggests a possible interaction of PXR with chromatin remodeling factor Sin3A [38]. It will be interesting to enquire whether these or other unknown players are also involved in PXR-mediated repression of AR.

How do we envision the function of PXR in normal and cancerous cells? Although the role of PXR is well defined in xenobiotic metabolism and elimination, recent research has revealed some undefined roles for PXR in modulating inflammation, lipid homeostasis, and cancer. A few recent reports on PXR upregulation in certain malignancies (colon, breast, endometrial, ovarian etc.) implicate its involvement in these cancerous conditions. Studies aimed at determining the significance of PXR expression in these malignancies are not clear and have provided conflicting results. There are reports that indicate PXR to be an inducer of cancer proliferation or implicate it in imparting increased metabolism and reduced clinical efficacy of some anticancer drugs [10–12]. Other reports also designate a protective role for PXR through repression of cancer proliferation especially in endocrine-related cancers [13,14]. In the present work, we have demonstrated PXR to be a potent repressor of AR signaling and have shown that PXR activators like rifampicin and methoxychlor down-regulate AR target genes i.e. PSA which is a well-established prognostic marker of prostate cancer progression. In view of the fact that AR plays a key role in prostate cancer progression and proliferation, the present observations suggest that AR–PXR cross-talk might play repressive role in prostate cancer conditions. However, the precise function of PXR in cancerous states needs to be clinically validated. Furthermore, when considering the involvement of ligand-activated PXR in prostate cancer, the association between PXR and anti-androgens/endocrine disrupting chemicals appears interesting. Although, the modulation of steroid receptors by these environmental compounds has been extensively studied, the role of other nuclear receptors such as xenobiotic receptors by anti-androgen/endocrine disruptor is yet to be defined. The present study suggests existence of an alternative mechanism of action of xenobiotics via cross-talk between AR and PXR pathways speculating a broader role of PXR in male reproductive physiology and therapeutics. Furthermore, since

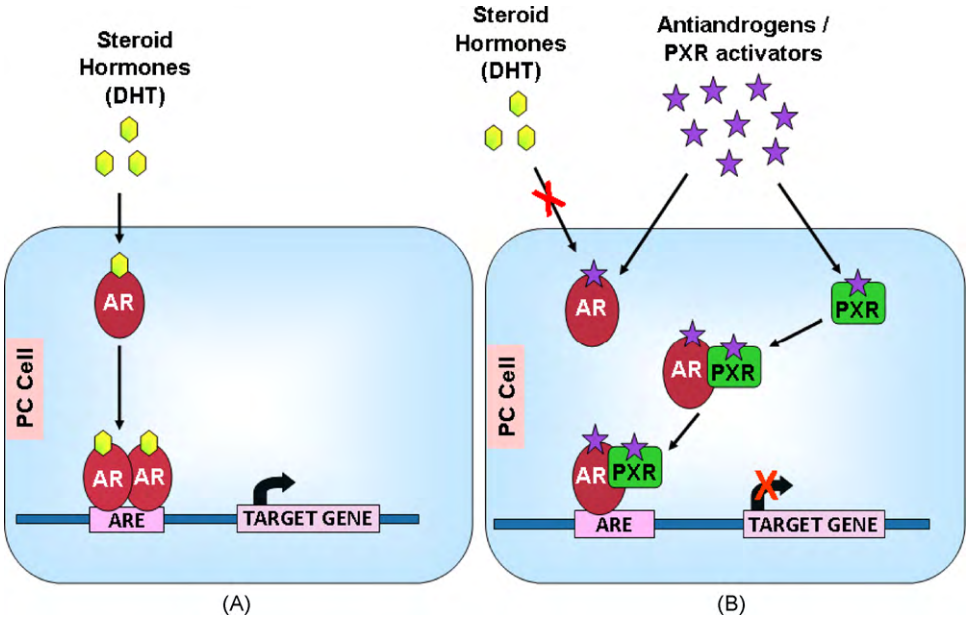

Fig. 10. A hypothetical model depicting an alternative mode of anti-androgen action. (A) Androgens such as DHT regulate the cellular process by binding to AR and inducing its nuclear translocation. The activated receptor can bind to specific androgen response elements (ARE) in the promoter regions of target genes, thereby triggering gene transcription. (B) PXR contributes to antagonist-mediated repression of AR activity. Competition with ligand for AR binding contributes to anti-androgen-mediated repression of AR activity. Based on our study we suggest a novel alternative mode of anti-androgen action. PXR acts as a molecular sensor for anti-androgens these compounds activate PXR. Activated PXR directly interacts with AR resulting in enhanced AR repression via anti-androgenic action.

AR–PXR interactions can be modulated by diverse ligands, the cues derived from such studies can be judiciously exploited in development of novel therapy in androgen-related diseases like prostate cancer and male infertility.

In brief, the highlight of the present study is the identification of a novel role of PXR as a potent repressor of AR signaling. We demonstrated PXR as a key deterministic component in anti-androgens action and displayed of a distinct antagonist-induced interaction with AR. In this perspective, the present study suggests an unidentified mode of action of AR antagonists through cross-talk between AR and PXR as described in a hypothetical model presented in Fig. 10. Alteration in AR–PXR stoichiometry may explain the failures in therapeutic regimen related to endocrine-mediated malignancies. This study may provide directions for the development of more efficacious AR antagonists towards the treatment of early stages of prostate cancer, and potentially advanced prostate cancer.

Conflict of interest statement

We do not have any conflict of interest including financial, personal or other relationships with other people or organizations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bcp.2010.06.009.

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