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Altered corepressor SMRT expression and recruitment to target genes as a mechanism that change the response to androgens in prostate cancer progression

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ABSTRACT

Androgen receptor (AR) is required for the development and progression of prostate cancer (CaP) from androgen-dependence to androgen-resistance. Both corepressors and coactivators regulate AR-mediated transcriptional activity, and aberrant expression or activity due to mutation(s) contributes to changes in AR function in the progression to androgen resistance acquired during hormonal ablation therapies. Primary culture of epithelial cells from androgen-dependent CWR22 and androgen-resistant CWR22R xenograft tumors were used to evaluate the effect of androgens on AR function, and the association with coactivators (SRC-1 and TIF-2) and corepressors (SMRT and NCoR). Both androgen-dependent CWR22 and androgen-resistant CWR22R cells expressed functional AR as the receptor bind ligand with high affinity and increased trafficking to the nuclei in the presence of androgens. However, in the presence of androgens, AR-mediated transcriptional activity in androgen-sensitive CWR22 cells was limited to a 2-fold increase, as compared to a 6-fold increase in androgen-resistance CWR22R cells. In androgen-sensitive CWR22 cells, immunoblot, confocal microscopy, and chromatin immunoprecipitation assays indicated that the androgen bound AR transcriptional initiation complex in the PSA promoter contained corepressor SMRT, resulting in limited receptor transcriptional activity. In contrast, increased AR-mediated transcriptional activity in the CWR22R cells was consistent with decreased expression and recruitment of the corepressors SMRT/NCoR, as well as increased recruitment of the coactivator TIF-2 to the receptor complex. Similar changes in the response to androgens were observed in the LNCaP/C4–2 model of androgen resistance prostate cancer. Thus, altered recruitment and loss of corepressors SMRT/NCoR may provide a mechanism that changes the response of AR function to ligands and contributes to the progression of the advanced stages of human prostate cancer.

1. Introduction

Androgen receptor (AR) mediates the biological effects of androgens and is involved in the development and progression of prostate cancer (CaP) to the androgen resistance [1]. In addition, AR is critical for recurrence during androgen deprivation bimodal therapies with GnRH agonists and androgen antagonist, such as Flutamide or Casodex [2,3]. Initially, androgen therapy is effective. However, CaP cells acquire the ability to grow despite the absence of circulating testicular androgens, and the disease progresses to an androgen-resistant stage that fails to respond to hormonal treatments [2,3].

Coactivators and corepressors regulate AR function by modulating the interactions between the receptor and the RNA pol II transcription initiation complex. AR corepressors include two highly related proteins, the silencing mediator for retinoid and thyroid hormone (SMRT) and the nuclear receptor corepressor (NCoR), among others [4,5]. AR coactivators include the steroid receptor coactivator (SRC's/p160) family of coregulators [6], the general coregulators CBP/p300 [7], and the AR associated (ARA) factors [8], among others [9]. Differential expression of AR-regulated genes in androgen-sensitive and androgen-resistant CaP may result from AR forming transcriptional complexes with different coregulators in the two stages of the disease.

This study evaluated the effects of coactivators SRC-1 and TIF-2, and corepressors SMRT and NCoR, on AR function, using primary cell

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cultures of androgen-dependent (CWR22) and androgen-resistant (CWR22R) tumor xenografts [10], and the LNCaP C4–2 cellular model of human prostate cancer to androgen independence. We demonstrate that the receptor level, affinity for androgens, and localization of androgen-bound AR are comparable in both cell types. However, AR activity in androgen-sensitive CaP is decreased due to increased SMRT recruitment to the AR transcriptional initiation complex in the presence of androgens, thereby limiting androgen-regulated gene expression. In contrast, SMRT recruitment to the AR transcriptional complex in the androgen-resistant CWR22 model of advanced prostate cancer is reduced. Similar changes in sensitivity to androgens for AR-mediated transcriptional activity was observed in the LNCaP/C4–2 model of advanced prostate cancer. Thus, decreased SMRT may represent the molecular switch for a mechanism that changes the androgen axis and gene expression during progression to androgen independence and resistance to hormonal therapies. Changes in coactivators and corepressors recruitment profiles to the AR are relevant when analyzing the effectiveness of antagonists in the hormonal treatment of advanced CaP.

2. Methods

2.1. Tumor digestion and primary cell culture

CWR22 and CWR22R xenografts were propagated in severe combined immunodeficient (SCID) mice. Tumor tissue specimens were cut in 3 mm pieces and digested 20 min in RPMI-1640 media containing 20% (v/v) fetal bovine serum (FBS) (Cellgro, Herndon, VA) and 0.1% (w/v) protease from *Streptomyces griseus* (Sigma–Aldrich, St. Louis, MO). CWR22 cells and CWR22R cells were collected by centrifugation, washed, and cultured 1–2 weeks in RPMI-1640 medium containing 10% (v/v) FBS and gentamicin (25 μg/ml, Cellgro).

2.2. Ligand-binding assays

CWR22 cells and CWR22R cells were pre-incubated 12 h in RPMI-1640 media containing 5% (v/v) charcoal-stripped FBS and used for binding assays with radiolabeled steroid. To calculate the dissociation constant (Kd) of AR for R1881, cells were incubated 4 h at 37 °C in increasing concentrations of [17α-methyl-^{3}H]-R1881 (Perkin Elmer, Boston, MA) ranging from 0 to 10 nM. Cells were washed and bound R1881 was extracted in ethanol, and released label measured using scintillation spectrometry (LS 6500 Multi-Purpose Scintillation Counter, Beckman Coulter, Fullerton, CA). The Kd for R1881 was determined using Scatchard analysis with Kd values representing the average of 3 independent experiments, each performed in triplicate.

2.3. Immunohistochemistry

Tissue from CWR22 and CWR22R xenograft tumors was formalin-fixed and paraffin-embedded for histological analysis. CWR22 and CWR22R cells from primary cell culture were fixed *in situ* 30 min at room temperature with 4% (w/v) paraformaldehyde. Histological sections and fixed cells were immunostained using anti-AR antibody PG21 (1:100, Upstate, Charlottesville, VA), anti-cytokeratin-18 (1:200, Santa Cruz Biotechnology, Santa Cruz, CA), anti-prostate specific antigen (PSA) (1:50, Abcam, Cambridge, MA), anti-smooth muscle actin (1:100, DakoCytomation, Carpinteria, CA), anti-vimentin (1:200, Santa Cruz Biotechnology), anti-desmin (1:200, Santa Cruz Biotechnology), or anti-SMRT (1:50, ABR, Golden, CO). HRP-conjugated anti-rabbit IgG or anti-mouse IgG (1:100, DakoCytomation) for peroxidase staining and Cy2 or Cy3-conjugated affinity-purified donkey anti-rabbit IgG (1:200, Jackson ImmunoResearch Laboratories, West Grove, PA) for immunohistochemistry and immunofluorescense were used as secondary antibodies, respectively. DAPI was used to visualize nuclei.

2.4. Luciferase assays

CWR22 and CWR22R cells in 48-well plates were transferred to RPMI-1640 media containing 5% (v/v) charcoal-stripped FBS (HighClone Laboratories, Logon, UT) and infected with column-purified adenoviral expression vectors for the luciferase reporter under the control of the mouse mammary tumor virus (MMTV) promoter or the PSA promoter (2.5 μl/ml, $\sim 10^{-9}$ adenoviral particles/ml, BD Adeno-X Virus Purification Kit, BD Biosciences, Palo Alto, CA). Luciferase activity was determined using the Luciferase Assay System (Promega) and Veritas microplate luminometer (Turner BioSystems, Sunnyvale, CA). Luciferase enzyme activity was normalized to total protein. All experiments were performed in triplicate at least three times.

2.5. Western immunoblotting

Proteins from cytosol and nuclear cell extracts (Pierce Biotechnology) (50 μg) were fractionated using 10% (w/v) SDS–polyacrylamide gel electrophoresis under reducing conditions and transferred to nitrocellulose membranes. Primary antibodies were anti-AR (1:1000, Upstate), anti-SRC-1 (1:2000, Upstate), anti-TIF-2 (1:500, Santa Cruz), anti-SMRT (1:250, ABR), anti-NCoR (1:250, Abcam), anti-β1-laminin (1:1:5000, Santa Cruz), or anti-α-tubulin (1:500, Sigma). Immunocomplex was visualized using enhanced chemoluminesence (Pierce Biotechnology).

2.6. Chromatin immunoprecipitation (ChIP)

For ChIP assays, CWR22 and CWR22R cells were infected 3 h with MMTV-luc adenovirus, maintained 24 h in RPMI-1640 media containing 5% (v/v) charcoal-stripped FBS, and treated 1 h with either vehicle (ethanol) or 5 nM R1881. Soluble chromatin was obtained after formaldehyde cross-linking and sonication. Chromatin fragments were immunoprecipitated overnight at 4 °C with specific antibodies. The immunoprecipitate was incubated 1 h with 20 μl of immobilized Proteins A- and G-agarose/salmon sperm DNA (Upstate). After washing, precipitates were eluted 1 h at room temperature with 1% (w/v) SDS and 0.1 M $NaHCO_3$. Reverse cross-linking was performed 4 h at 65 °C in high concentrations of NaCl, followed by proteinase K treatment 1 h at 45 °C. Peptides were removed from the DNA solution using phenol–chloroform extraction. PCR reactions were performed using Platinum PCR Super Mix 96 (Invitrogen, Carlsbad, CA), 2 μl DNA, and 34 cycles of amplification. Primers for the MMTV promoter were: forward primer (GGTTACAAACTGTTCTTAAAACGAGG), and reverse primer (CAGAGCTCAGATCAGAACCTTTGA). PCR product was resolved on 10% polyacrylamide gel and visualized with ethidium bromide.

3. Results

3.1. Characterization of primary cell cultures isolated from CWR22 and CWR22R xenografts

Short-term primary cell cultures of CWR22 and CWR22R were established from xenografts tumors grown in SCID hosts. Tumor cells in xenografts and in primary cultures were positive for the expression of epithelial markers AR, cytokeratin-18 and PSA, and negative for stromal markers vimentin and desmin, indicating that primary cell cultures contain epithelial cells and accurately represent the cellular population found within the xenograft *in vivo* (Fig. 1). Ligand binding activity assays indicated that AR in both cell

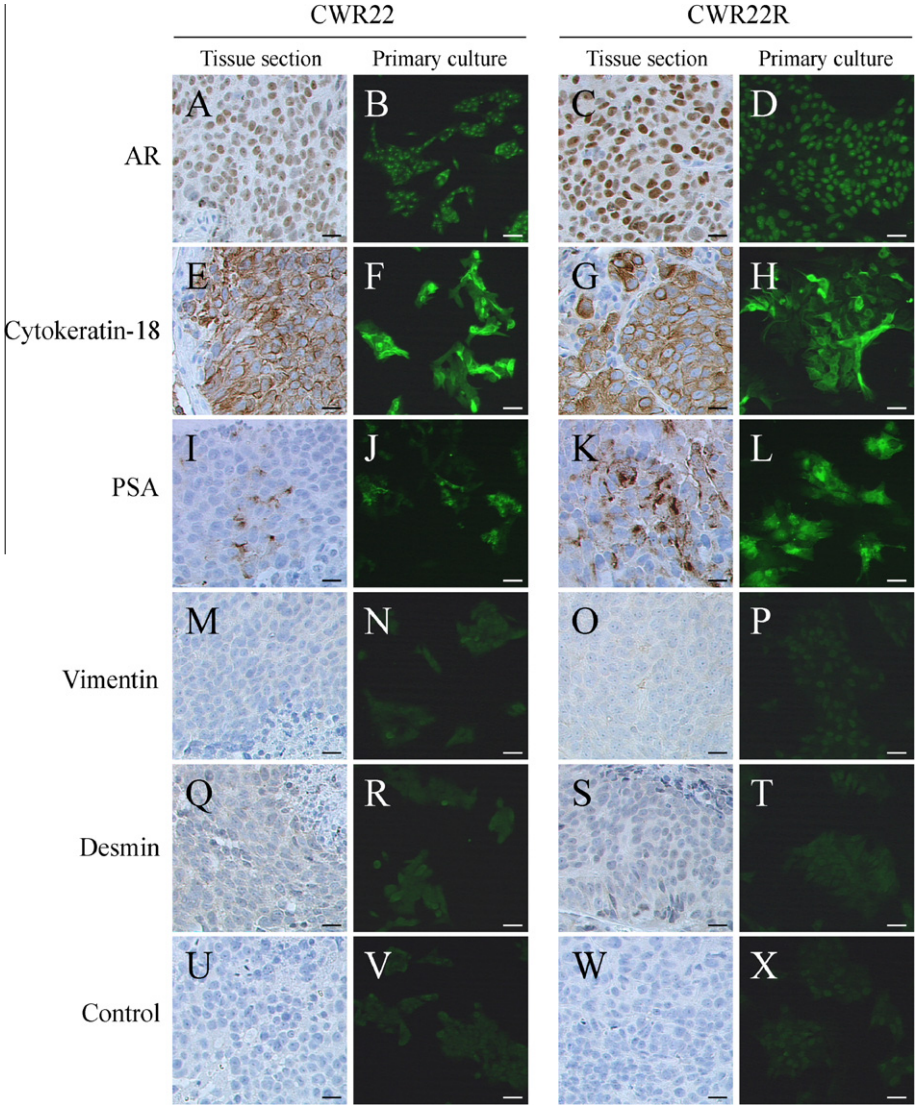

Fig. 1. Immunostaining analysis of epithelial and stromal markers in CWR22 and CWR22R cells. Tissue sections of CWR22 and CWR22R tumors and primary cell cultures of CWR22 and CWR22R xenografts were analyzed for the expression of AR (A–D), cytokeratin-18 (E–H), PSA (I–L),vimentin (M–P), and desmin (Q–T). Tissue sections and primary cell cultures of CWR22 and CWR22R showed expression of AR, cytokeratin-18, and PSA. Immunostaining for vimentin and desmin was negative in tissue sections and primary cell cultures. Negative controls (U–X) included immunostaining assays without primary antibody or with nonspecific IgGs. Black bars: 10 μm, white bars: 20 μm.

types bound radiolabeled androgen agonist R1881 (5 nM) that was displaced from the receptor with 100 nM of unlabeled R1881, dihydrotestosterone, or estradiol, but not with 100 nM of unlabeled dexamethasone or 1;25 dihydroxycholecalciferol (Fig. 2A). The R1881 binding to AR increased in a dose-dependent manner and saturated at ligand concentrations of 4 nM for CWR22 cells and 8 nM for CWR22R cells (Fig. 2B). Scatchard analysis indicates a single ligand binding site for AR in both cell types, with a Kd of 0.46 nM and 1.45×10^8 receptors/μg protein in CWR22 cells, and a Kd of 0.95 nM and 2.7×10^8 receptors/μg protein in CWR22R cells (Fig. 2C). Ligand dependent cytosol–nuclear trafficking of AR was monitored in both cell types using immunofluorescence with an AR specific antibody. In the absence of R1881, AR protein localized primarily to cytoplasm of CWR22 cells (Fig. 2D). In contrast, unliganded AR was localized in the nuclei in CWR22R cells (Fig. 2I). In the presence of R1881, AR localized to the nuclei of both CWR22 (Fig. 2E, F) and CWR22R cells (Fig. 2J, K). Thus, the receptor level, affinity for androgens, and localization of androgen-bound AR are comparable in both cell types. In addition, CWR22R expresses slightly more AR, which is localized in the nucleus in the absence or presence of androgens (Fig 2C, F, K).

3.2. Increased AR-mediated transcriptional activity in androgen resistant CWR22R cells

AR-mediated transcriptional activity in CWR22 and CWR22R cells was analyzed using luciferase expression vector driven by Mouse Mammary Tumor Virus (MMTV-Luc) or PSA (PSA-Luc) promoters. Transfection efficiency of the reporter constructs in CWR22 and CWR22R cells was evaluated using an adenovirus encoding green fluorescent protein (Ad-GFP, 10 μl/ml) under the control of a CMV promoter. Similar infection efficiencies were obtained in both cell types, and at least 85% of the cells expressed GFP after 36 h of infection (Data not shown). R1881 and DHT stimulated MMTV-Luc and PSA-Luc activity in both cell types in a dose-dependent manner (Fig. 3A). Androgens-induced MMTV-Luc reporter activity was increased 2–3-fold in CWR22 cells, and 6–10-fold in CWR22R cells, compared to the reporter activity in the absence of ligand (Fig. 3B). Similarly, PSA-Luc reporter activity was increased 1.5-fold in CWR22 cells and 3-fold in CWR22R cells (Fig. 3C). Both basal and ligand-induced transcriptional activities were higher in CWR22R than CWR22 with the MMTV-Luc (6-fold) and with the PSA-Luc (3.5-fold) reporters (Fig. 3B, C). The increased

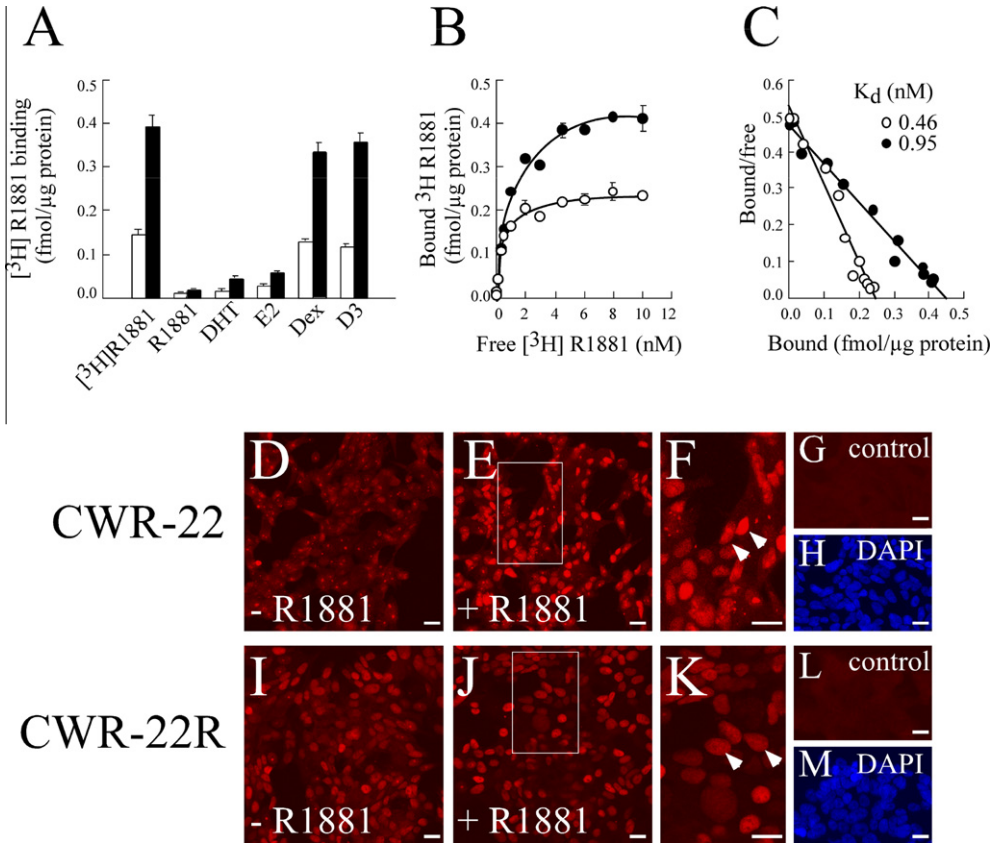

Fig. 2. R1881 binding activity and nuclear translocation of AR in CWR22 and CWR22R primary cell cultures. (A) AR-ligand binding specificity was determined in CWR22 cells (□) and CWR22R cells (■) using competition assays with 5 nM [$_3$H] R1881 binding competed by excesses (100 nM) of unlabeled R1881 (R1881), dihydrotestosterone (DHT), estradiol (E2), dexamethasone (Dex) or 1;25 dihydroxycholecalciferol (D3). (B) AR binding saturation analyses were performed by incubating CWR22 cells (○) and CWR22R cells (●) with [^{3}H] R1881 at concentrations from 0.01 to 10 nM. (C) Scatchard plot of the binding data from (B) indicated a single binding site with dissociation constants (Kd) of 0.46 for CWR22 (○) and 0.95 nM for CWR22R (●). (D–F) Immunofluorescence detection of nuclear translocation of AR in CWR22 cells in the absence (D) and presence (E, F, arrows) of R1881. (I–K) Immunofluorescence detection of nuclear translocation of AR in CWR22R cells in the absence (I) and presence (J, K, arrows) of R1881. (G, H, L, M) Negative controls for immunofluorescence studies for CWR22 cells (G, H) and CWR22R cells (L, M) were incubated in the absence of primary antibody. DAPI (blue) was used to detect nuclei (H, M). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

AR transcriptional activity in CWR22R could not be explained by differences in AR expression levels between the two cell types. Western immunoblotting using antibody against AR with the whole cell lysate from the androgen dose response experiment indicated AR levels in CWR22 in the presence of androgens were equivalent or greater than in CWR22R cells (Fig. 3D). Interestingly, consistent with the number of receptors determined in the ligand binding assays, CWR22R in the absence of androgen stimulation only expressed 1.7-fold more AR than in CWR22 cells (Fig. 2D). Altogether, these results indicate that AR-mediated transcriptional activity is increased in the CWR22R, compared to AR activity CWR22 (Fig.3A–C). Studies of AR-mediated transactivation in the androgen-sensitive LNCaP and in the androgen-resistant LNCaP-derived C4–2 cell lines of human prostate cancer demonstrate that the receptor is activated by androgens in the sub-nanomolar range in C4–2 and required higher concentrations of androgens to detect activation of the reporters in LNCaP despite similar AR expression in both cell types (not shown).

3.3. Increased TIF-2 and diminished SMRT and NCoR expression are associated with increased AR-mediated transcriptional activity in CWR22R cells

Expression levels and sub-cellular distributions of AR coactivators SRC-1 and TIF-2, and corepressors SMRT and NCoR, were determined in CWR22 and CWR22R cells using immunoblot analyses of cytoplasmic and nuclear cell extracts. Consistent with immune histochemistry analysis, AR was found in the cytosolic fraction in CWR22 cells, in the absence of R1881 treatment. Substantial AR levels were found in nuclear extracts of CWR22R cells (Fig. 3E). In the presence of hormone, AR was primarily found in the nuclear fraction of both CWR22 and CWR22R cells.

SRC-1 was found exclusively in the nuclear fraction in both cell types, with increased levels in CWR22 (Fig. 3E). In contrast, the TIF-2 was found in both the nuclear and cytoplasmic fractions, and at increased levels in CWR22R cells (Fig. 3E). R1881 treatment had no significant effect on expression of either SRC-1 or TIF-2 at the protein level (Fig. 3E). SMRT and NCoR also demonstrated cell type-specific patterns of expression. SMRT and NCoR were detected at increased levels in CWR22, compared to CWR22R cells (Fig. 3E). SMRT was found exclusively in the nuclear fraction of CWR22 cells, but was undetectable in CWR22R cells (Fig. 3E). NCoR expression was also increased in the nuclear fraction of CWR22. Incubation with R1881 had no significant effects on SMRT and NCoR expression (Fig. 3E). Consequently, increased levels of AR-mediated transactivation observed in CWR22R compared to CWR22 cells correlated with increased TIF-2 expression, and diminished SMRT and NCoR expression.

3.4. Changes in corepressors and coactivators recruitment are responsible for altered AR-mediated transcriptional activity in CWR22 and CWR22R cells

The components of the AR transcriptional complex were examined using ChIP assay to identify SMRT and NCoR present within

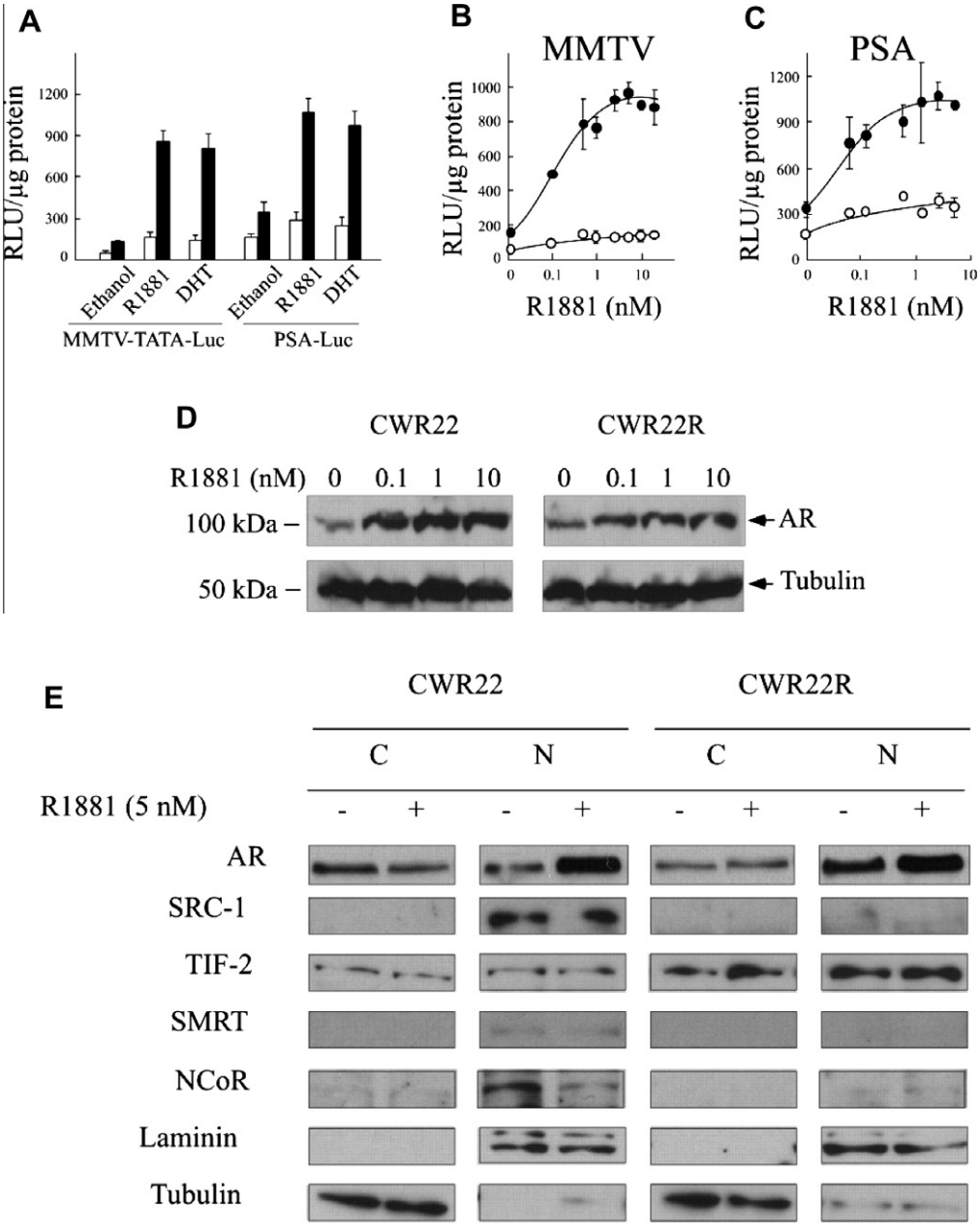

Fig. 3. AR-mediated transcriptional activity and expression in CWR22 and CWR22R primary cell cultures. (A) Relative luciferase reporter activity driven by MMTV and PSA promoter were determined in CWR22 cells (□) and CWR22R cells (■) in the presence of 5 nM R1881 or DHT. (B) Dose–response analysis of R1881 in CWR22 cells (○) and CWR22R cells (●) using a luciferase reporter driven by the MMTV promoter. (C) Dose–response analysis of R1881 in CWR22 (○) and CWR22R (●) using a luciferase reporter driven by the PSA promoter. (D) Immunoanalysis of AR in western blots of CWR22 cells and CWR22R cells treated with 0, 0.1, 1, and 10 nM R1881. (E) Infection efficiency in CWR22 (□) and CWR22R (■) cells was determined by calculating the percent of GFP adenovirus-infected cells (10 µl/ml, $\sim 10^{-10}$ adenoviral particles/ml) compared to total cells determined by DAPI staining. For each condition, more than 10 fields were analyzed. (E) Cellular distribution of AR, SRC-1, TIF-2, SMRT and NCoR. CWR22 cells and CWR22R cells were exposed for 1 h to vehicle (−, ethanol) or 5 nM R1881 and Nuclear (N) and cytoplasmic (C) cellular extracts were prepared using the NE-PER kit (Pierce Biotechnology). Membranes and nuclei were probed with antibodies against AR, SRC-1, TIF-2, SMRT, NCoR. β1-laminin (nuclear), or α-tubulin (cytoplasmic) were used as loading controls.

the complex, in the presence and absence of androgen. As expected, more AR was localized to the MMTV promoter in both cell types when androgens were present (Fig. 4A). However, in the absence of hormone in CWR22R cells, more AR was bound to the MMTV promoter compared to CWR22 cells (Fig. 4A). This result was consistent with increased nuclear staining for AR in CWR22R cells in the absence of hormone (Fig. 2). In addition, consistent with increased AR transcriptional activity in CWR22R cells, SRC-1 and TIF-2 were increased, and SMRT and NCoR were decreased within the AR transcriptional complex in the presence of androgens. Reflecting the decreased transcriptional activity found in CWR22 compared to CWR22R, androgens failed to promote efficient recruitment of SRC-1 and TIF-2. SMRT and NCoR were present within the AR transcriptional complex in CWR22 cells (Fig. 4A). Consistent with increased recruitment of SMRT to the AR transcriptional complex, more SMRT was co-localized with ligand-bound AR in the nucleus of CWR22 than CWR22R cells (Fig. 4B–I). In summary, decreased AR mediated transcriptional activity in androgen-dependent CWR22 cells correlated with decreased coactivator and increased corepressor recruitment. Increased AR mediated transcriptional activity in androgen-resistant CWR22R cells correlated with recruitment of TIF-2 and diminished recruitment of

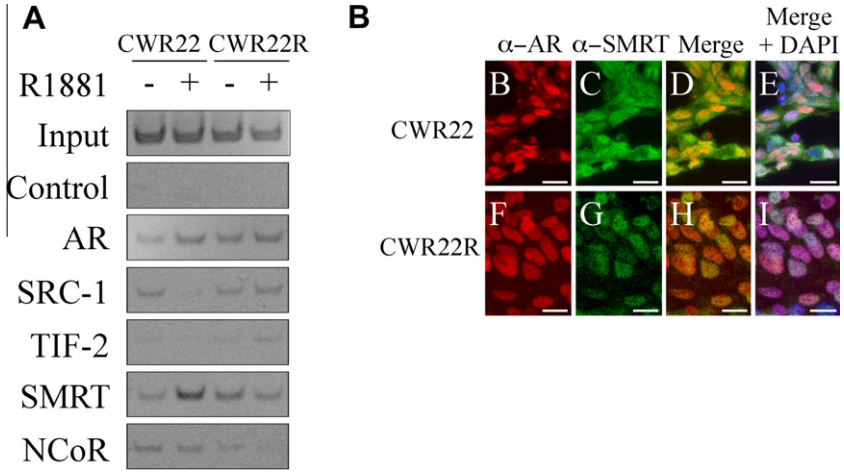

Fig. 4. Coregulator recruitment and colocalization to AR complex in CWR22 cells and CWR22R cells. (A) CWR22 cells and CWR22R cells transfected with the MMTV driven reporter construct were treated with ethanol vehicle or R1881 for 45 min, and ChIP assays were performed using primers specific for the MMTV-LTR. The elevated transcriptional activity observed in CWR22R cells correlated with increased recruitment of SRC-1 and TIF-2 and diminished recruitment of NCoR and SMRT to the AR transcriptional complex. Rabbit or mouse normal serum was used as negative control. (B) AR and SMRT co-localization was analyzed using confocal laser scanning microscopy in CWR22 cell (B–E) and CWR22R cells (F–I) in the presence of 5 nM R1881. AR was visualized with Cy3-conjugated (Red) antibody and SMRT was visualized with Cy2-conjugated (Green) antibody. DAPI (Blue) was used to detect nuclei. Bars: 10 μm.

SMRT and NCoR to the AR transcriptional complex in the presence of androgens. Thus, the presence of corepressors decreases gene transcriptional activity in androgen-dependent cells, and the presence of coactivators shifts the balance towards enhanced gene transcriptional activity in the androgen-resistant cell types. Similar to our findings with CWR22 and CWR22R cells, SMRT expression was reduced in clinical samples of androgen-resistant CaP [11]. These results indicated that decreased SMRT expression observed in the CWR22 model and loss of AR corepressors is, at least in part, relevant in the progression of prostate cancer to androgen independence.

4. Discussion

Although key components of the AR transcriptional complex on androgen regulated genes have been described in detail, little is known about the role of coactivators and corepressors during progression to androgen resistance in CaP [12]. Coactivators, including SRC-1/TIF-2, CBP/p300, pCAF, and the chromatin remodeling SWI/SNF proteins form high-order complexes that possess intrinsic histone acetyltransferase (HAT) activity [13,14] to remodel chromatin structure for better access of the receptor to the promoter, and to allow full transcriptional capacity. Corepressors SMRT/NCoR are related structurally, and are also components of a multi-subunit protein complex that represses the transcriptional activity of either unliganded, agonist-bound or antagonist-bound AR by recruitment of histone deacetylases (HDACs) to the promoter [15,16]. Possible pathways for CaP recurrence include AR gene amplification; increased growth factor-induced phosphorylation; stabilization of AR; mutations that change hormone specificity; intracrine production of androgens; and altered expression and interaction with coactivators to increase transactivation [17]. Increased expression of coactivators has been implicated in the increase of AR transactivation in response to low circulating androgens [17]. Coactivators, ARA55, CBP, and ARA70, were altered in prostate cancer cell lines [17]. The SMRT/NCoR corepressors were shown to interact directly with AR in the absence and presence of antagonists to repress AR transcriptional activity in LNCaP cells [15,16,18]. Thus, the alteration of the relative ratio between corepressors and coactivators recruitment to AR is a potential mechanism to change the androgen signaling axis during CaP progression to androgen independence.

The kinetic parameters of androgen binding to AR in the two cell lines used suggests that the elevated AR-mediated transcriptional activity in CWR22R cells was not due to cell line specific differences intrinsic to AR, but rather differences in other components of the AR-mediated transcriptional complex. The affinity of AR for R1881 was only slightly higher in CWR22 than CWR22R cells, thus the increased sensitivity to androgens of the androgen-resistant cells cannot be explained solely by an increased affinity for ligand. Levels of DHT in tissue specimens from androgen-resistant CaP have averaged about 1.5 nM, suggesting that AR could be reactivated fully in androgen-resistant prostate cancer [19]. Ligand-induced nuclear translocation of AR was observed in both cell types. However, CWR22R cells demonstrated high nuclear immunostaining for AR in the absence of added ligand. Therefore, nuclear AR in absence of androgens could represent an additional phenotype of androgen resistance in CaP.

The increased level of AR-mediated transactivation observed in androgen resistant CWR22R cells correlated with decreased protein expression and recruitment of corepressors SMRT/NCoR. In contrast, the increased recruitment of SMRT in CWR22 cells suggests that the corepressor is involved in ligand-dependent suppression of AR activity. Increased recruitment of SMRT, in conjunction with diminished recruitment of SRC-1/TIF-2 in androgen-dependent CaP cells appears to maintain AR activity at low basal level. When tumor cells progress to androgen resistance, the decreased recruitment of the corepressors SMRT/NCoR, together with increased recruitment of SRC-1/TIF-2 coactivators, reactivates but alters AR function. Consistently, decreased SMRT expression has been observed in leukemias, lymphomas, and androgen-resistant CaP [11,20,21]. Therefore, loss of corepressors, in conjunction with increased expression and recruitment of the coactivators, provide a mechanism for increased AR activity that is more independent of ligands in androgen-resistant CaP. Furthermore, changes in HDAC enzyme activity of corepressors SMRT/NCoR at the promoter DNA-elements in androgen-regulated genes may characterize androgen sensitivity and resistance to hormonal therapies in CaP.

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