Biochemical and Biophysical Research Communications 412 (2011) 13–19

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Biochemical characterization of nuclear receptors for vitamin D_3 and glucocorticoids in prostate stroma cell microenvironment

Alejandro A. Hidalgo [a,b], Viviana P. Montecinos [a], Roberto Paredes [a], Alejandro S. Godoy [a], Eileen M. McNerney [a], Heribelt Tovar [a], Diego Pantoja [a], Candace Johnson [b], Donald Trump [c], Sergio A. Onate [a,d,*]

[a] Laboratory of Molecular Endocrinology, Department of Physiopathology, University of Concepcion, Concepcion, Chile
[b] Department of Molecular Pharmacology and Therapeutics, NY, USA
[c] Department of Medicine, Roswell Park Cancer Institute, Buffalo, NY, USA
[d] Department of Urology, State University of New York at Buffalo, NY, USA

ARTICLE INFO

Article history:
Received 28 June 2011
Available online 5 July 2011

Keywords:
Vitamin D receptor
Glucocorticoids receptor
Coactivators
Corepressors
Prostate cancer
Cellular microenvironment
Transcription regulation

ABSTRACT

The disruption of stromal cell signals in prostate tissue microenvironment influences the development of prostate cancer to androgen independence. 1α,25-Dihydroxyvitamin D_3 ($1,25D_3$) and glucocorticoids, either alone or in combination, have been investigated as alternatives for the treatment of advanced prostate cancers that fails androgen therapies. The effects of glucocorticoids are mediated by the intracellular glucocorticoid receptor (GR). Similarly, the effect of $1,25D_3$ is mediated by the $1,25D_3$ nuclear receptor (VDR). In this study, fibroblasts from benign- (BAS) and carcinoma-associated stroma (CAS) were isolated from human prostates to characterize VDR and GR function as transcription factors in prostate stroma. The VDR-mediated transcriptional activity assessed using the CYP24-luciferase reporter was limited to 3-fold induction by $1,25D_3$ in 9 out of 13 CAS (70%), as compared to >10-fold induction in the BAS clinical sample pair. Expression of His-tagged VDR (Ad-his-VDR) failed to recover the low transcriptional activity of the luciferase reporter in 7 out of 9 CAS. Interestingly, expression of Ad-his-VDR successfully recovered receptor-mediated induction in 2 out of the 9 CAS analyzed, suggesting that changes in the receptor protein itself was responsible for decreased response and resistance to $1,25D_3$ action. Conversely, VDR-mediated transcriptional activity was more efficient in 4 out of 13 CAS (30%), as compared to the BAS sample pair. Consistent with the reduced response to $1,25D_3$ observed in CAS, chromatin immunoprecipitation (ChIP) assays indicated decreased recruitment of coactivators SRC-1/CBP, without major changes in the recruitment of VDR to the CYP24 promoter. In addition, we observed that GR-mediated transcriptional activity was also altered in CAS, as compared to BAS. Disruption of coactivators SRC-1/CBP recruitment may promote hormone resistance in CaP, and highlights the relevance of molecular diagnosis and drug design in tumor cell microenvironment.

1. Introduction

Growth factors produced in prostate stroma regulate proliferation and differentiation of the glandular epithelia, in an androgen-dependent manner [1]. Changes in stromal-epithelial cell interactions during carcinogenesis generate a cellular microenvironment that fosters local tumor growth, invasion and progression to androgen independence [2]. Fibroblasts found in the stroma of invasive tumors are known as reactive stroma, and are involved in the paracrine activity of growth factors and proteases that alter cell growth and extracellular matrix to facilitate invasion [2]. Additional steroid hormones, including 1α,25-dihydroxyvitamin D_3 ($1,25D_3$) and glucocorticoids, are important modulators of the stromal-epithelial cell signaling interactions in the prostate [3,4].

Prostate tumor cells, over time, acquire the ability to grow in the presence of blockade testicular and adrenal androgens. Clinical trials demonstrate that alternative treatment options, at this stage, are limited [3]. Calcitriol, the active form of vitamin D, either alone or in combination with glucocorticoids, has been investigated for the mitigation of prostate cancer progression that acquires the ability to grow, despite the androgen blockade. The $1,25D_3$ potential as an anticancer drug is mediated by the intracellular vitamin D receptor (VDR), and includes the induction of cell-cycle arrest, differentiation and apoptosis, as well as decreased invasiveness and

* Corresponding author at: Laboratory of Molecular Endocrinology, Department of Physiopathology, University of Concepcion, Concepcion, Chile. Fax: +56 41 220 6465.
E-mail address: sergio.onate@udec.cl (S.A. Onate).

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.06.181

angiogenesis in several tumor models [3,4]. High VDR expression in prostate tumors is associated with reduced lethality in advanced prostate cancer [4]. However, potential benefits of high-dose treatment with $1,25D_3$ in patients with advanced prostate cancer were hampered by development of hypercalcemia resulting from changes in calcium and phosphate metabolism [3].

Glucocorticoids have been used in clinical trials to treat this induced hypercalcaemia, in order to decrease intestinal absorption of calcium, increase renal secretion, and decrease synthesis of adrenal androgens in response to the negative feed-back mechanism on the pituitary–adrenal axis. The intracellular glucocorticoids receptor (GR) may mediate a direct effect on cellular signals that relates to induction of programmed cell death and decreased expression of cytokines and angiogenic factors, thus becoming relevant to the potential treatment of advanced stages [3,4]. However, the development of glucocorticoids resistance in cancer cells also limits its therapeutic use.

Regulation of transcriptional programs by VDR and GR requires receptor interacting proteins, identified as corepressors and coactivators, to silence or increase the expression of hormonally regulated genes [5]. The first corepressors identified were the Silencing Mediator for Retinoid and Thyroid hormone (SMRT) and the Nuclear Receptor Corepressor (NCoR) [10]. The binding of hormone induces the release of the corepressors and recruitment of coactivators to achieve full receptor transcriptional capacity [5]. Classic coactivators for nuclear receptor is the Steroid Receptor Coactivator-1 (SRC-1/p160) family of proteins [6], and the general CBP/p300/pCAF [7]. The intrinsic histone acetyl-transferase activity present in this coactivator complex enables chromatin remodeling for better access of the RNA pol II pre-initiation complex to activate transcription [5,8].

In this study, expression of GR and VDR was characterized in prostate cells isolated from BAS and CAS microenvironment. Short-term cultures expressed the stroma cell-specific markers Vimentin, Desmin, and smooth muscle actin. Both cell types expressed GR and VDR in the nuclear compartment and activated the expression of target genes in a ligand-specific manner. However, the ability of GR and VDR to drive gene expression differed in CAS compared to BAS. Reduced VDR-mediated transcriptional activity in CAS was consistent with decreased recruitment of SRC-1 and CBP coactivators to the VDR complex. Decreased SRC-1/CBP coactivators recruitment may result in hormone resistance to glucocorticoids and $1,25D_3$ in stromal cell microenvironment during cancer progression. Characterization of coregulator profile in prostate stroma microenvironment may provide novel perspectives in the treatment of prostate cancer, as well as other malignancies.

2. Materials and methods

2.1. Isolation of stromal cells from prostate

Prostate tissue was obtained from the Translational Research Tissue Support Resource at Roswell Park Cancer Institute under Internal Review Board approved protocols. Prostatectomy specimens are procured in the pathology core, and classified as normal, hyperplastic, prostatic intraepithelial neoplasia (PIN) or cancer. The remaining pathology core tissue, otherwise discarded, was use in this study. Stromal derived cells were obtained from 14 patients scheduled for radical prostatectomy. Prostate tissues from the malignant sample and benign counterpart were subjected to collagenase type I digestion, and short-term prostatic primary cell cultures were maintained in 10% FBS containing media for 4–5 passages, as described [9]. After passage 4 and 5, cultures were above 95% enriched in fibroblast-like stromal cells. Primary cell culture at these early passages can be routinely cryopreserved liquid N_2, and recovered with 95% viability [9]. For this study, BAS and CAS were grown in parallel and used between passages 4 and 5.

2.2. Ligand-binding assays

BAS and CAS cells were pre-incubated overnight in RPMI-1640 media containing 5% (v/v) charcoal-stripped FBS and used for binding assays with radio-labeled [^{3}H]-dexamethasone. To calculate the dissociation constant (*Kd*) of GR for dexamethasone, cells were incubated 4 h at 37 °C in increasing concentrations of [^{3}H]-dexamethasone (Perkin Elmer, Boston, MA) ranging from 0 to 10 nM. Cells were washed three times with ice-cold PBS, bound R1881 was extracted in ethanol, and released label measured using scintillation spectrometry. The *Kd* for dexamethasone was determined using Scatchard analysis with *Kd* values representing the average of three independent experiments, each performed in triplicate. To calculate the hormone binding activity for VDR in BAS and CAS, cells were pre-incubated overnight in RPMI-1640 media containing 5% (v/v) charcoal-stripped FBS and used for binding assays with radio-labeled [^{3}H]-$1,25D_3$ at 1 mM in absence and 10-fold excess of unlabeled ligand. Bound [^{3}H]-$1,25D_3$ was extracted in ethanol and measured using scintillation spectrometry as above (LS 6500 Multi-Purpose Scintillation Counter, Beckman Coulter, Fullerton, CA).

2.3. VDR and GR immunostaining

Stromal cells grown on glass slides chambers (Nalge Nunc, Naperville, IL) for 24 h were incubated in DMEM containing 10% dextran-charcoal treated FBS for hormone withdrawal prior to treatment with 50 nM of $1,25D_3$ or ethanol vehicle for 1 h at 37 °C. For GR analysis, cells were treated with 5 nM Dexamethasone, or as indicated in figure legends. Cells were fixed with 4% formaldehyde, and immunostained for VDR using mouse monoclonal anti human VDR antibody (D6) (1/500 diluted; Santa Cruz Biotechnology, Santa Cruz, CA), or normal rabbit IgG as a control (1/500 diluted, SC-2027; Santa Cruz Biotechnology, Santa Cruz, CA). Donkey anti-mouse Cy3 conjugated (1/1000 diluted; Chemicon, Temecula, CA) was used as secondary antibody. Nuclei were detected using DAPI staining [18]. Anti-human GR antibody was used for GR immunostaining (H-300, Santa Cruz Biotechnology INC, Santa Cruz, CA) or anti-GR (Affinitty Bioreagents Inc, Golden, Co).

2.4. Adenoviral Expression Vectors

Adenoviral expression vectors encoding the luciferase reporter under the control of the CYP24 promoter and encoding for wild type human VDR and GR, were produced in HEK-293QB1 cells by co-transfecting cells with the pBHGlox(delta)E1,3 Cre adenoviral genomic DNA, and either pDC311-CYP24-luciferase reporter or the His-tagged wild type VDR adenoviral expression shuttle vectors (Microbix, Ontario, Canada). The resulting adenoviral expression vectors expressing CYP24-luciferase (Ad-CYP24-luc) and the human VDR (Ad-his-VDR) were isolated and titer by DNA content, as recommended by manufacturer protocols (Microbix, Ontario, Canada). Luciferase reporter assay was determined using 2×10^4 BAS or CAS cells in absence and presence of $1,25D_3$, from 10^{-12} to 10^{-6} M final concentrations [9].

2.5. Chromatin Immunoprecipitation (ChIP) assay

ChIP assay was used to determine VDR and coregulator recruitment of corepressor SMRT and coactivator SRC-1 to the CYP24 promoter [9]. Stromal cells grown in 10% charcoal-striped FBS were

infected with Ad-CYP24-Luc reporter for 3 h at 37 °C in CO_2 incubator for 36 h and treated with hormone for 1 h at 37 °C. Cells were cross-linked with 1% formaldehyde for 10 min and soluble chromatin obtained using sonication. Fifty micrograms of protein extract was used for immunoprecipitation with specific antibodies, as indicated in the Figure legends. Immunoisolated complexes were incubated for 4 h at 65 °C to reverse cross-linking and immune isolated DNA fragments containing the VDR transcriptional complexes amplified using PCR with primers specific for the CYP24 promoter primers (forward: cgttggaagcacacccggtg; reverse: ccaccccggagataaccccc). PCR product was fractionated onto PAGE 5–20% gradient, and visualized using ethidium bromide staining.

3. Results

3.1. Characterization of primary cultures of human prostatic BAS and CAS cells

Prostate stromal cells in primary cell cultures were positive for vimentin and desmin, indicating the presence of fibroblast in the isolated stroma (Fig. 1A). Expression of smooth muscle actin (SMA) indicated the presence of myoblast in primary cell cultures (Fig. 1A). Other stromal cell markers previously reported in our laboratory include KGF, FGF2, ps20 and vimentin [9]. In addition, cell cultures were negative for epithelial cell markers (PSA and cytokeratin 18) after passage three. Thus, cell cultures were enriched for fibroblasts and myoblast present in the cell population of the prostate stroma.

3.2. Characterization of GR and VDR in BAS and CAS

Ligand dependent cytoplasmic-nuclear trafficking of GR and VDR was monitored in both BAS and CAS cell using immunofluorescence with receptor specific antibodies. In the absence of added glucocorticoids, GR protein was localized in the cytoplasm in BAS and CAS cells (Fig. 1B). The addition of glucocorticoids localized GR to the nuclei in both cell types (Fig. 1B). In contrast, VDR expression was nuclear, in the absence and presence of added $1,25D_3$ ligand in BAS and CAS cells (Fig. 1B). The presence of GR and VDR in the nuclear compartment suggests functional receptor. However, specific transcription assays are required to demonstrate that GR and VDR are working as transcription factors in isolated BAS and CAS cells.

3.3. Functional characterization of GR and VDR in BAS and CAS

The ability of GR to activate gene expression in BAS and CAS was characterized using an adenoviral expression vector for the luciferase reporter under the control of the MMTV hormone responsive DNA element (Ad-MMTV-Luc). The MMTV is a classic and well-characterized hormone-responsive DNA-element (HRE) model system used to study steroid hormone-mediated gene transcription. Cells from BAS and CAS were grown and infected with Ad-MMTV-Luc in the absence or presence of increasing concentrations of synthetic glucocorticoid agonist, dexamethasone (Fig. 2A). Basal transcriptional activity of the Ad-MMTV-Luc reporter was enhanced by dexamethasone in BAS and CAS cell types. However, GR-mediated transcriptional activity of the luciferase reporter was limited to 1–2-fold in 4 out of 10 (40%) CAS, as compared to 5–6-fold increase in BAS (Fig. 2A). Resistance to glucocorticoids may reside in altered structural/functional domains of the GR protein present in CAS, resulting in decreased transactivation. We reasoned that co-infection of adenoviral expression vectors encoding wild type GR should revert the reporter activity in CAS. An adenovirus expression vector encoding the nuclear-targeted β-galactosidase enzyme activity (Ad-β-gal) was used to determine that the relative infection efficiencies in primary cell cultures was present in at least 95% of the cells. The adenoviral expression vector encoding wild type GR (Ad-his-GR) and the Ad-MMTV-Luc reporter were

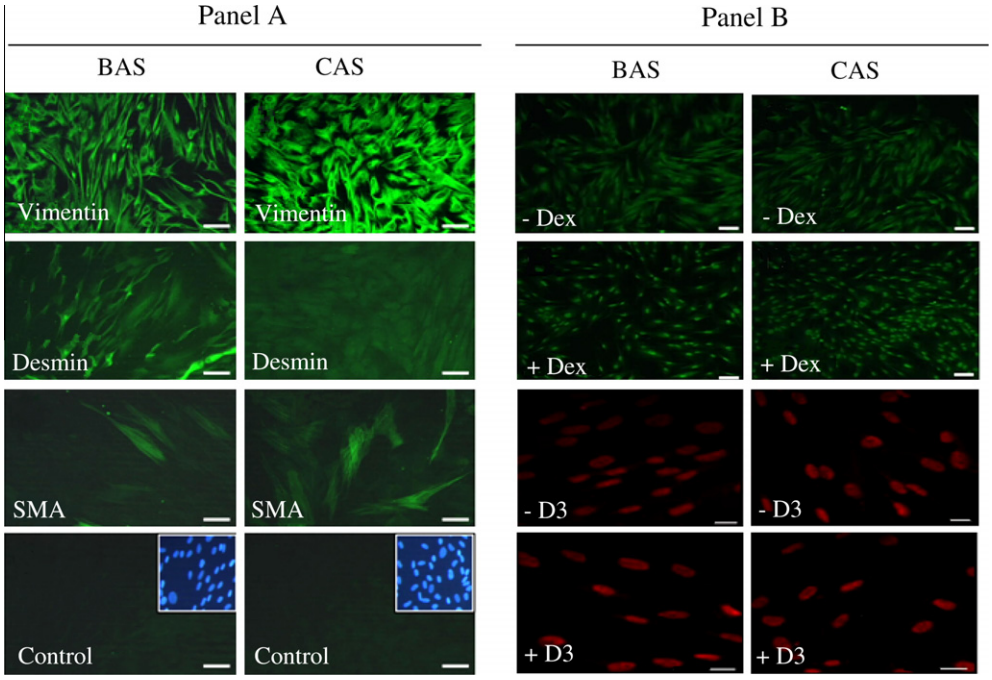

Fig. 1. Immunostaining analysis of stromal markers and nuclear receptors in BAS and CAS cells. (A) Paraformaldehyde-fixed primary cell cultures of BAS and CAS were analyzed for expression of stromal-specific cell markers Vimentin, Desmin, and Smooth Mucle Actin as indicated. Immunostaining was negative for PSA and cytekeratin 18. The inset indicates nuclei staining with DAPI. (B). Immune fluorescence detection of nuclear translocation of GR and VDR in primary cell cultures of BAS and CAS were analyzed in absence and presence of Dexamethasone (10 nM) and $1,25D_3$ (10 nM), as indicated. Positive nuclear staining indicates expression nuclear of GR and VDR in presence of their cognate hormone.

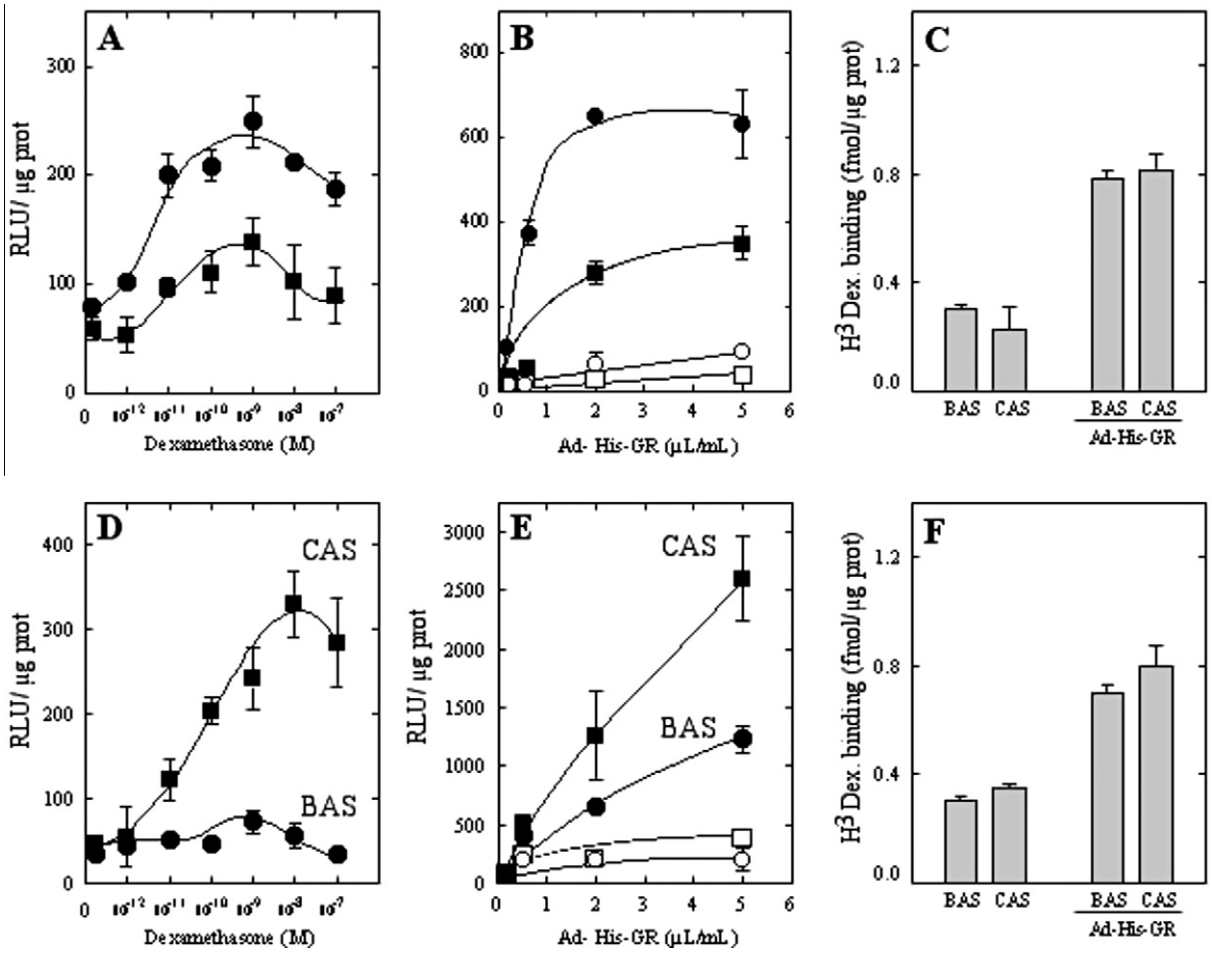

Fig. 2. GR-mediated transcriptional activity in BAS and CAS isolated from different prostates BAS and CAS cells isolated from prostate samples, representing two different responses to glucocorticoids, are indicated in panels (i) A–C and (ii) D–F. Isolated cells from each prostate sample were infected with Ad-MMTV-Luc alone (A and D), or in combination with Ad-his-GR (B and E). The MMTV-luciferase reporter activity driven by endogenous GR was determined in absence and presence of increasing concentrations of Dexamethasone, as indicated (A and D). The MMTV-luciferase reporter activity driven by exogenous Ad-his-GR was determined in absence and presence of increasing concentrations of adenoviral particles (Ad-his-GR) in cells exposed to Dexamethasone at 10 nM (filled circles) or vehicle control (empty circles), as indicated (B and F). Relative [^{3}H]-Dexamethasone-binding activity determined relative expression of endogenous and exogenous His-tagged GR in BAS and CAS (C and F).

use to co-infect BAS and CAS, in the absence or presence of dexamethasone (Fig. 2B). The expression of Ad-his-GR resulted in a ligand-dependent increased luciferase reporter in BAS and CAS, as compared to endogenous GR. However, exogenous GR transactivation of the reporter in CAS remained limited, as compared to BAS (Fig. 2B). Radioligand binding assays using [^{3}H]-dexamethasone demonstrated that the expression of GR was similar in BAS and CAS (Fig. 2C). Interestingly, GR-mediated transcriptional activity of the MMTV-luciferase reporter in CAS cells isolated from a different clinical samples, was more efficiently induced by dexamethasone in a concentration-dependent manner, as compared to the reporter activity in BAS (Fig. 2D). Exogenous Ad-his-GR resulted in a ten-fold increase of luciferase reporter in both cell types, and GR maintained higher transcriptional capacity of the reporter in CAS as compared to BAS (Fig. 2E), despite similar levels of receptor expression (Fig. 2F). This highlights the potential role of accessory proteins that affect GR efficiency.

The VDR-mediated transcriptional activity in BAS and CAS was determined using the adenoviral expression vector encoding the luciferase reporter under the control of the CYP24 promoter. Basal transcriptional activity of the Ad-CYP24-Luc reporter was enhanced by 1,25D$_3$ in BAS and CAS. However, VDR-mediated transcriptional activity was limited to 1–2-fold in 8 out of 14 (57%) CAS, as compared to 10–20-fold increase in BAS. Differences in VDR transactivation was observed at increasing concentrations of 1,25D$_3$, from 10^{-12} to 10^{-6} M (Fig. 3A). Co-infection of Ad-CYP24-Luciferase and an adenoviral expression vector encoding His-tagged-VDR (Ad-his-VDR) resulted in increased reporter activity in BAS and CAS (Fig. 3B). The exogenous Ad-his-VDR trans-activation of the reporter remained limited in 5 out of 8 CAS, as compared to BAS. Hormone binding assays using [^{3}H]-1,25D$_3$ and Western immunoblotting demonstrated VDR expression in BAS and CAS (Fig. 3C and D, respectively). Expression of Ad-his-VDR in 3 out of 8 CAS that exhibited limited VDR transactivaation compared to BAS (Fig. 3E), efficiently recovered reporter activity in a ligand-dependent manner (Fig. 3F). Hormone binding and Western immunoblot demonstrate VDR expression in BAS and CAS (Fig. 3G and H). Therefore, resistance to 1,25D$_3$ in these 3 clinical samples may reside in altered structural/functional domains of VDR protein in CAS, as compared to BAS (Fig. 3E and F). In addition, VDR-mediated transcriptional activity induced by hormone was observed to be more efficient in 3 out of 14 CAS, as compared to BAS (Fig. 3I). Overexpression of the receptor increased VDR-mediated transactivation in CAS and BAS (Fig. 3J). However, differences in the luciferase reporter activity, observed between CAS and BAS, continued after expression of exogenous VDR (Fig. 3J). Hormone-

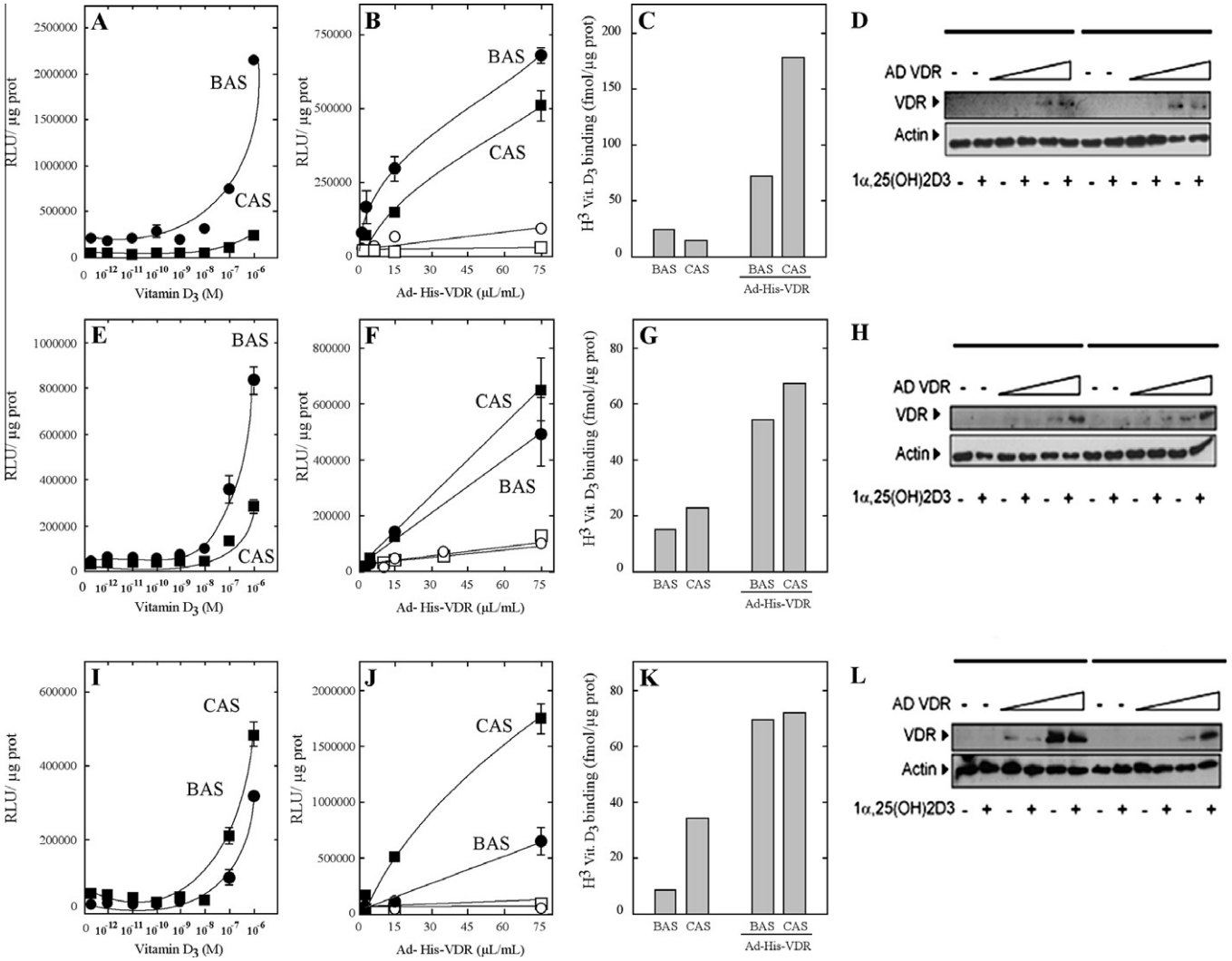

Fig. 3. VDR-mediated transcriptional activity in BAS and CAS isolated from different prostates BAS and CAS cells isolated from prostate samples representing three different responses to $1,25D_3$, are indicated in panels (i) A–D, (ii) E–H, and (iii) I–L. Isolated cells from each of the three prostate samples were infected with Ad-CyP24-Luc alone (A, E and I) or in combination with Ad-his-VDR (B, F and J). The CyP24-luciferase reporter activity by endogenous VDR was determined in absence and presence of increasing concentrations of $1,25D_3$, as indicated (A, E and I). The CyP24-luciferase reporter activity driven by exogenous Ad-his-VDR was determined in absence (empty circles) and presence (filled circles) of $1,25D_3$ at 10 nM final concentration using increasing concentrations of Ad-his-VDR expression vector, as indicated (B, F and J). Relative $[^3H]$-$1,25D_3$-binding activity in the three clinical samples (C, G and K) of endogenous VDR and exogenous Ad-his-VDR was determined in BAS and CAS, as indicated. Immune detection of VDR expression in total cell extracts of the luciferase reporter assays was determined using Western Immunoblot and VDR-specific antibodies in three prostate samples (D, H and L). The expression of VDR and actin are indicated.

binding activity and Western immunoblotting assays demonstrated VDR expression in BAS and CAS (Fig. 3 K and L). In summary, differences in VDR-mediated reporter activity were observed in BAS and CAS from 14 prostate clinical samples.

3.4. Altered recruitment of VDR coactivators SRC-1 and CBP

The recruitment of VDR, coactivators SRC-1/CBP, and corepressor SMRT to the CYP24 promoter in BAS and CAS, isolated from two prostate samples was analyzed using ChIP assays and specific antibodies in the absence and presence of added $1,25D_3$ (Fig. 4). As expected, VDR is recruited to the CYP24 promoter in absence and presence of hormone in BAS and CAS (Fig. 4A and B). The presence of $1,25D_3$ increased recruitment of VDR and coactivators SRC-1/CBP (Fig. 4A and B), and decreased recruitment of SMRT corepressor in BAS and CAS cells (Fig. 4A and B). However, recruitment of coactivators SRC-1 and CBP in CAS was reduced, as compared to BAS (Fig. 4A and B). These results support the concept that changes in the recruitment of nuclear receptors and coregulators to the promoter region in hormonally regulated target genes in prostate cancer microenvironment may contribute to altered response mechanisms to agonist and antagonists during cancer progression.

4. Discussion

Numerous gene expression studies, requiring relatively few cultured prostatic cells, can be performed using adenoviral expression vectors and luciferase reporter. Adenovirus has the advantage of both replicating and non-replicating cells in primary cultures that can be infected with high efficiency, and the gene product analyzed a few hours after infection. For these studies, the adenoviral delivery system was used to determine the ability of VDR and GR to activate gene expression, elucidating differences in the ability to work as transcription factors in the various clinical samples. This suggests that,

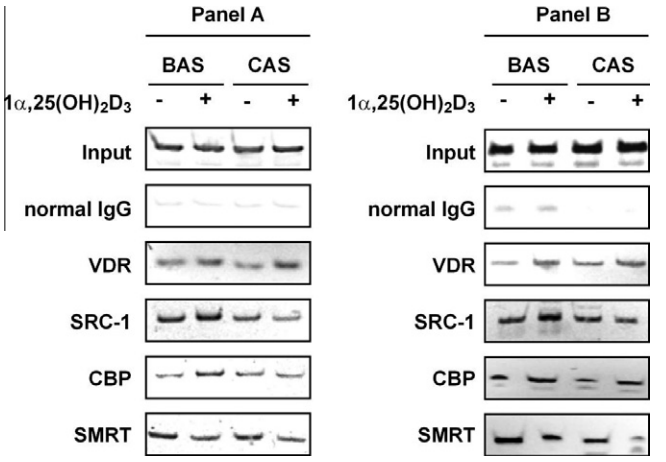

Fig. 4. Coregulator recruitment to VDR complex in BAS and CAS cells. BAS and CAS cells representing two prostate samples with reduced VDR-mediated transactivation in CAS, as compared to BAS, were analyzed for VDR and coregulator recruitment to the CyP24 promoter using ChIP assays. Isolated BAS and CAS cells were incubated with CyP24-luciferase reporter and then treated for 1 h with ethanol vehicle (−) or 1,25D_3 (+). ChIP assays were performed using antibodies specific for VDR, SRC-1 and CBP coactivators, and SMRT corpressor, with PCR primers for the CyP24 promoter. The reduced transcriptional activity observed in CAS cells correlated with decreased recruitment of SRC-1 and CBP to the VDR transcriptional complex. An aliquot of the chromatin complexes before immunoisolation was used as input (10%). Rabbit or mouse normal serum was used as negative control.

as the samples analyzed were essentially primary localized tumors with Gleeson scores of 3 + 4, the development of 1,25D3 and glucocorticoid resistance in prostate cellular microenvironment may occur at an early stage in tumor progression, rather than in late metastatic stages. An expanded clinical trial is needed to further determine the molecular mechanism for early development of hormone resistance in prostate cancer microenvironment.

In vivo and in vitro studies indicate that circulating 1,25D_3 is a protective factor for prostate cancer, and represents a potent agent for the inhibition of tumor progression due to the effect on cellular signals that increase cell-cycle arrest, differentiation and apoptosis [3,4]. High VDR expression in prostate tumors is associated with reduced risk of lethal phenotype. VDR gene polymorphisms that result in reduced protein expression also heighten the risk of prostate cancer. However, the significance of receptor protein variations in clinical samples, including single nucleotide polymorphism, is relatively uncertain. In our studies, the use of adenoviral expression vectors for GR and VDR in BAS and CAS identified whether hormone resistance to either glucocorticoids or 1,25D_3 was due to changes in the receptor structure itself, or in corepressors and coactivators, that are required for full transcription capacity.

Several coactivators, including SRC-1, CBP, and chromatin remodeling SWI/SNF factors, form high-order complexes that remodel chromatin structure for receptor access to the promoter region of hormone regulated target genes [10–12]. A specific coactivator complex for the thyroid hormone receptor-associated protein (TRAP) [13], and its homologue VDR interacting protein (DRIP) [13], is involved in coactivation and corepression of VDR and GR. Deregulation of these coregulators, including SRC-1, RAC3/IAB1, and CBP, have been associated with the development of several cancers [5,14–17]. Increased expression of coactivators SRC-1, TIF2, IAB1/RAC3, CBP, ARA55 and ARA70 is found in prostate cancer epithelial cells and cell lines [14–19]. However, the role of these coregulators in the prostate cancer cellular microenvironment is limited [15]. This report indicates that recruitment of coactivators SRC-1 and CBP are important for proper VDR transcriptional activity in prostate stromal cell microenvironment. The failure of some isolated CAS to efficiently respond to 1,25D_3 is due, at least in part, to decreased recruitment of coactivators SRC-1 and CBP to the VDR transcriptional complex in the CYP24 promoter, without the occurrence of major changes in receptor recruitment. Therefore, we speculate that the development of glucocorticoid and other hormone resistance, observed in clinical samples, is also due to altered coregulator recruitment to the GR complex. The critical role of prostate stromal cell microenvironment in normal and disease progression is supported by clinical observations and tissue recombination experiments [20,21]. The development of a model to identify the role of corepressors and coactivators in prostate cancer cellular microenvironment is necessary to elucidate the interaction of AR, GR and VDR signaling pathways in tumor progression.

Acknowledgments

This study was supported by the Fondecyt (1080261), American Cancer Society (RGS-012301-TBE), and the Concern Foundation to Sergio A. Onate. The authors would like to acknowledge the support from the Translational Research Tissue Resource and the Pathology core facilities at Roswell Park Cancer Institute for providing prostate tissue from the organ donor program.

References

[1] R.A. Taylor, G.P. Risbridger, Prostatic tumor stroma: a key player in cancer progression, Curr. Cancer Drug Targets 8 (2008) 490–497.
[2] S. Josson, Y. Matsuoka, L.W. Chung, H.E. Zhau, R. Wang, Tumor-stroma coevolution in prostate cancer progression and metastasis, Semin. Cell Dev. Biol. 21 (2010) 26–32.
[3] M.K. Chadha, L. Tian, T. Mashtare, V. Payne, C. Silliman, E. Levine, M. Wong, C. Johnson, D.L. Trump, Phase 2 trial of weekly intravenous 1,25 dihydroxy cholecalciferol (calcitriol) in combination with dexamethasone for castration-resistant prostate cancer, Cancer 116 (2010) 2132–2139.
[4] I. Chung, W.D. Yu, A.R. Karpf, G. Flynn, R.J. Bernardi, R.A. Modzelewski, C.S. Johnson, D.L. Trump, Anti-proliferative effects of calcitriol on endothelial cells derived from two different microenvironments, J. Steroid Biochem. Mol. Biol. 103 (2007) 768–770.
[5] B.W. O'Malley, R. Kumar, Nuclear receptor coregulators in cancer biology, Cancer Res. 69 (2009) 8217–8222.
[6] S.A. Onate, S.Y. Tsai, M.J. Tsai, B.W. O'Malley, Sequence and characterization of a coactivator for the steroid hormone receptor superfamily, Science 270 (1995) 1354–1357.
[7] A.J. Bannister, T. Kouzarides, The CBP co-activator is a histone acetyltransferase, Nature 384 (1996) 641–643.
[8] C.K. Glass, M.G. Rosenfeld, The coregulator exchange in transcriptional functions of nuclear receptors [In Process Citation], Genes Dev. 14 (2000) 121–141.
[9] P. Cano, A. Godoy, R. Escamilla, R. Dhir, S.A. Onate, Stromal-epithelial cell interactions and androgen receptor-coregulator recruitment is altered in the tissue microenvironment of prostate cancer, Cancer Res. 67 (2007) 511–519.
[10] B. York, B.W. O'Malley, Steroid receptor coactivator (SRC) family: masters of systems biology, J. Biol. Chem. 285 (2010) 38743–38750.
[11] H.w. Chen, R.j. Lin, R.l. Schiltz, D. Chakravarti, A. Nash, L. Nagy, M.l. Privalsky, Y. Nakatani, R.m. Evans, Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with PCAF and CBP/p300, Cell 90 (1997) 569–580.
[12] T.E. Spencer, G. Jenster, M.M. Burcin, C.D. Allis, J.X. Zhou, C.A. Mizzen, N.J. McKenna, S.A. Onate, S.Y. Tsai, M.J. Tsai, B.W. Omalley, Steroid receptor coactivator-1 is a histone acetyltransferase, Nature 389 (1997) 194–198.
[13] K.J. Stanya, H.Y. Kao, New insights into the functions and regulation of the transcriptional corepressors SMRT and N-CoR, Cell Div. 4 (2009) 7.
[14] J.X. Zou, Z. Zhong, X.B. Shi, C.G. Tepper, R.W. deVere White, H.J. Kung, H. Chen, ACTR/AIB1/SRC-3 and androgen receptor control prostate cancer cell proliferation and tumor growth through direct control of cell cycle genes, Prostate 66 (2006) 1474–1486.
[15] E.Y. Hsia, M.L. Goodson, J.X. Zou, M.L. Privalsky, H.W. Chen, Nuclear receptor coregulators as a new paradigm for therapeutic targeting, Adv. Drug Deliv. Rev. 62 (2010) 1227–1237.
[16] A.B. Johnson, B.W. O'Malley, , Steroid receptor coactivators 1, 2, and 3: Critical regulators of nuclear receptor activity and steroid receptor modulator (SRM)-based cancer therapy, Mol. Cell Endocrinol. (2011).
[17] A.L. Kung, V.I. Rebel, R.T. Bronson, L.E. Ch'ng, C.A. Sieff, D.M. Livingston, T.P. Yao, Gene dose-dependent control of hematopoiesis and hematologic tumor suppression by CBP, Genes Dev. 14 (2000) 272–277.

[18] B. Comuzzi, C. Nemes, S. Schmidt, Z. Jasarevic, M. Lodde, A. Pycha, G. Bartsch, F. Offner, Z. Culig, A. Hobisch, The androgen receptor co-activator CBP is up-regulated following androgen withdrawal and is highly expressed in advanced prostate cancer, J. Pathol. 204 (2004) 159–166.
[19] C. Nessler-Menardi, I. Jotova, Z. Culig, I.E. Eder, T. Putz, G. Bartsch, H. Klocker, Expression of androgen receptor coregulatory proteins in prostate cancer and stromal-cell culture models, Prostate 45 (2000) 124–131.
[20] IG. Schauer, DR. Rowley, The functional role of reactive stroma in benign prostatic hyperplasia, Differentiation (2011 June 9).
[21] A. Planche, M. Bacac, P. Provero, C. Fusco, M. Delorenzi, JC. Stehle, I. Stamenkovic, Identification of prognostic molecular features in the reactive stroma of human breast and prostate cancer, PLoS One 6 (2011) e18640.