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Bone stroma-derived cells change coregulators recruitment to androgen receptor and decrease cell proliferation in androgen-sensitive and castration-resistant prostate cancer cells

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ABSTRACT

Prostate cancer (CaP) bone metastasis is an early event that remains inactive until later-stage progression. Reduced levels of circulating androgens, due to andropause or androgen deprivation therapies, alter androgen receptor (AR) coactivator expression. Coactivators shift the balance towards enhanced AR-mediated gene transcription that promotes progression to androgen-resistance. Disruptions in coregulators may represent a molecular switch that reactivates latent bone metastasis. Changes in AR-mediated transcription in androgen-sensitive LNCaP and androgen-resistant C4-2 cells were analyzed for AR coregulator recruitment in co-culture with Saos-2 and THP-1. The Saos-2 cell line derived from human osteosarcoma and THP-1 cell line representing human monocytes were used to display osteoblast and osteoclast activity. Increased AR activity in androgen-resistant C4-2 was due to increased AR expression and SRC1/TIF2 recruitment and decreased SMRT/NCoR expression. AR activity in both cell types was decreased over 90% when co-cultured with Saos-2 or THP-1 due to dissociation of AR from the SRC1/TIF2 and SMRT/NCoR coregulators complex, in a ligand-dependent and cell-type specific manner. In the absence of androgens, Saos-2 decreased while THP-1 increased proliferation of LNCaP cells. In contrast, both Saos-2 and THP-1 decreased proliferation of C4-2 in absence and presence of androgens. Global changes in gene expression from both CaP cell lines identified potential cell cycle and androgen regulated genes as mechanisms for changes in cell proliferation and AR-mediated transactivation in the context of bone marrow stroma cells.

1. Introduction

Androgen receptor (AR) is essential for the development and progression of prostate cancer (CaP) from an androgen-sensitive state to a castration-resistant phenotype [1]. This progression involves metastasis of CaP cells to various sites, including bone, lung, liver, pleura and adrenals, with bone involvement presenting the most frequently [2]. Approximately 85% of CaP mortality cases demonstrate bone metastasis [3]. Although once considered a linear progression, recent research suggests that bone metastasis is an early event in CaP progression.

The intracellular effects of androgens are mediated by the androgen receptor (AR) [4]. Coactivators are required for receptors to achieve full transcriptional capacity through the recruitment of histone acetyl- and methyl-transferase activities to remodel chromatin at the transcription initiation site [5]. Corepressors SMRT and

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NCoR represses gene transcription due to recruitment of histone deacetylases to the promoter of target genes [6]. These coregulators play a fundamental role in modifying pathways that are conducive to tumor development.

Disruption of androgen-regulated pathways in the context of stromal-epithelial cell interactions are required for CaP initiation and progression to androgen resistance and metastasis [7,8]. With progression, coactivators (SRC-1/TIF-27RAC3 and p300,/CBP) are overexpressed and corepressor SMRT reduced, resulting in increased AR transactivation in response to lower circulating androgens [9,10]. This altered expression may result in AR transcription complexes with different coregulators, thereby explaining the failure of androgen deprivation bimodal therapies.

Bone involvement may be detected as early as Gleason 2 and is reactivated due to changes in circulating androgens levels, AR alterations, and crosstalk between fibroblast growth factors and bone stroma cells [11,12]. AR-induced changes in the bone stroma osteolytic microenvironment promotes sclerotic lesions through the induction of osteoblast proliferation [13]. The bone microenvironment affects CaP cell proliferation through soluble factors from bone matrix or osteoblasts [14]. Reciprocal interactions are fundamental for CaP progression and bone metastasis reactivation [15]. This study evaluated the effects of bone stroma-derived Saos-2 and THP-1 on AR function, SRC-1/TIF-2 and SMRT/NCoR recruitment to AR complex, cell proliferation and changes in the global gene expression in androgen-sensitive LNCaP and androgen-resistant LNCaP/C4-2B (hereafter termed C4-2) cells as tumor models of human CaP progression to androgen independence and metastasis. AR activity in CaP cells was decreased when co-cultured with Saos-2 or THP-1 due to dissociation of the AR complex from SRC1/TIF2 and SMRT/NCoR, in a ligand-dependent and bone marrow-derived cell-type specific manner. Analysis of global changes in gene expression from LNCaP and C4-2 in combination with Saos-2 or THP-1 identified relevant mRNA expression signatures as potential mechanisms for changes in cell proliferation and AR-mediated transcription activity.

2. Methods

2.1. Cell cultures

LNCaP, C4-2, and THP-1 cells were propagated in RPMI-1640 media supplemented with 5% fetal bovine serum (FBS), as described [8,10]. Saos-2 were cultured in DMEM media supplemented as above. Cells cultured alone or in co-cultures were seeded at 80% confluence in a 24 or 12 well-format plates using the Transwell™ insert of 0.4 μm (Costar, Cambridge, MA). Cell cultures were pre-incubated for 24 h in phenol red-free RPMI/DMEM mix media (1:1 ratio) containing 5% (v/v) of charcoal-coated dextran-stripped (cd) FBS (Cellgro, Herndon, VA) and supplements [8,10].

2.2. Adenoviral transfections and Luciferase reporter assays

CaP cells were infected with adenoviral expression vectors for Luciferase reporter Ad-PSA-Luc or Ad-MMTV-Luc [8] for 2 h in a CO_2 incubator [10]. For co-culture, Saos-2 or THP-1 were added directly to CaP cells or to the Transwell™. Relative Luciferase reporter (RLU) activity was determined after 36 h of infection [10].

2.3. Immunohistochemistry staining and flow cytometry assays

LNCaP and C4-2 were fixed *in situ* and immunostained using antibodies for AR and coregulators indicated. HRP-conjugated anti-rabbit IgG and FIT-C, Cy2, Cy3, or Cy5-conjugated IgG were used as secondary antibodies. Imaging captures were obtained using confocal microscopy [10]. Mitotic index flow cytometry assays (Abcam, Cambridge, MA) using Fluor 488 conjugated anti Histone 3 pSer10-H3 antibody (pS10-H3) in combination with propidium iodine incorporation (P.I.I.) coupled to fluorescence activated cell scanning (FACS) were used to determine mitotic index in co-culture with Saos 2 or THP-1 in the Transwell™. At least 2×10^4 events of labeled cells were used to determine the placement of the gates and analyzed using FACS, according to manufacturer conditions (Accuri C6, BD Biosciences, Franklyn Lakes, NY).

2.4. Chromatin immunoprecipitation (ChIP) assays

LNCaP and C4-2 (2×10^6) cells were infected 3 h with Ad-PSA-Luc expression vector, either alone on in co-culture with Saos-2 or THP-1 using the Transwell™ and ChiP assay for PSA promoter occupancy performed as described [8,10].

2.5. Microarray scanning and screening for differential gene expression

LNCaP or C4-2 were cultured alone or with Saos-2 or THP-1 using the Transwell™ insert for 36 h at 37 °C in a CO_2 incubator in absence or presence of androgens. Total RNA (250 ng) was isolated using TRIzol one-step and reverse transcribed to cRNA using biotin-UTP labeled nucleotide and Illumina® TotalPrep RNA Amplification Kit (Ambion, Foster City, CA). Biotinylated cRNAs were hybridized to the Illumina Human-ref12 V3.0 BeadChip (Illumina, San Diego, CA). Arrays were scanned in Illumina BeadArray Reader and relative gene expression was determined using fold change (FC) by subtracting the intensity signal of CaP in co-culture with Saos-2 or THP-1, from the intensity signal of LNCaP or C4-2 cultured alone. The ratio between the subtracted signals provided the FC in mRNA expression for each gene in CaP cells. Genes with FC $\geq |1.9|$ were considered significant and subjected to Venn diagrams.

3. Results

3.1. Decreased SMRT/NCoR expression and recruitment increased AR-mediated transcription in androgen-resistant CaP cells

AR-mediated transcriptional activity in LNCaP and C4-2 was analyzed using Luciferase expression vector driven by the Ad-MMTV-Luc or Ad-PSA-Luc promoters. Androgens (DHT) stimulated PSA-Luc and MMTV-Luc activity in both cell types (Fig. 1A). Androgen-induced PSA-Luc reporter activity was increased 3–5-fold in LNCaP and 6–10-fold in C4-2, compared to the reporter activity in the absence of ligand (Fig. 1A). The MMTV-Luc reporter activity was increased 10-fold in LNCaP, and 20–30-fold in C4-2 (Fig. 1A). The mutated AR (T877A) activated by estrogens also increased PSA-Luc reporter in C4-2 than in LNCaP cells exposed to estradiol in the absence and presence of Tamoxifen (Fig. 1A). Higher AR-mediated transcription activity in C4-2 was observed with increasing concentration of androgens and saturated at 1 nM for C4-2 and 10 nM for LNCaP (Fig. 1B). Transfections using adenovirus encoding green fluorescent protein demonstrated similar infection efficiencies in both cell types (data not shown). Western immunoblot assays indicated that AR levels were increased in C4-2, as compared to AR in LNCaP, in absence and presence of androgens (Fig. 1C). Subcellular distributions of AR, SRC-1, TIF-2, SMRT and NCoR using Western immunoblot of cytosol and nuclear extracts showed increased AR levels in nuclear extracts of both cell types in the presence of hormone, with more AR in C4-2 (Fig. 1C). SMRT and NCoR expression was reduced in C4-2 (Fig. 1D–E). The NCoR was detected exclusively in the nuclear fraction of LNCaP cells (Fig. 1F). SRC-1 and TIF2 were in the nuclear fraction with similar levels in both cell types. Androgens

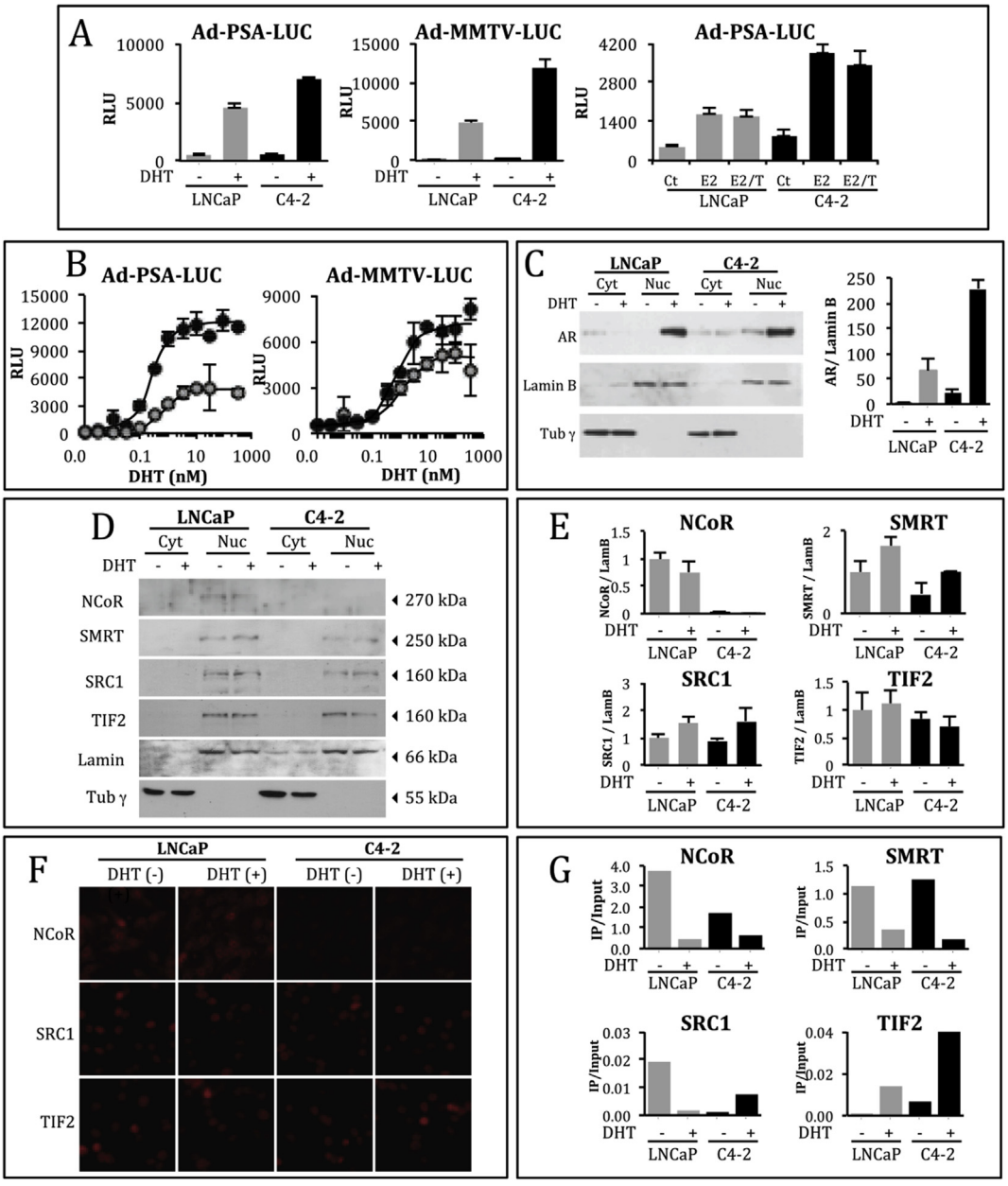

Fig. 1. Increased AR-mediated transcription activity in androgen-resistant C4-2 cells. (A) Luciferase reporter activity driven by the PSA promoter (Ad-PSA-LUC) or MMTV promoter (Ad-MMTV-LUC) in LNCaP (▩) or C4-2 (■) cells was determined in the absence (−) and presence (+) of 10 nM of DHT, estradiol (E2) and estradiol with Tamoxifen (E2/T). (B) Luciferase reporter activity was determined in LNCaP (●) and C4-2 (●) with increasing concentration of DHT (0–1 μM). (C) Protein from cytosol (Cyt) and nuclear (Nuc) extracts obtained in absence (−) and presence (+) of 10 nM DHT using the NE-PER were analyzed for AR expression using Western immunoblot. Lamin B1 and γtubulin (γTub) were used as controls. Relative densitometric values provide AR expression (right panel). (D) Protein extracts were used to determine expression of NCoR, SMRT, SRC1 and TIF2. (E) Densitometric analysis of the immune detected protein complex are indicated. (F) Representative images of immunofluorescence signal obtained using confocal microscopy of NCoR, SRC1 and TIF2 in absence (−) or presence (+) of 10 nM of DHT. Cy3 conjugated secondary antibodies were used to detect the primary immune complex. (G) The AR coregulator recruitment to the PSA promoter in LNCaP (▩) and C4-2 (■) was determined in the absence (−) and presence (+) of 10 nM DHT using ChIP assays with antibodies against NCoR, SMRT, SRC-1 and TIF2 and specific primers for the PSA promoter.

had no major effect on the coregulator expression at the protein level (Fig. 1D–F). ChIP assays demonstrated that SMRT and NCoR were within the AR complex in the absence of androgens (Fig. 1G). Androgens released the receptor from SMRT and NCoR complex in both cell types (Fig. 1G); and more TIF2 and SRC-1 was found within the androgen-bound AR complex in C4-2 (Fig. 1G).

3.2. Saos-2 and THP-1 change AR coregulator recruitment in CaP cells

The Ad-PSA-Luc reporter activity in LNCaP and C4-2 was analyzed in the absence and presence of androgens, either alone or in co-culture with Saos-2 or Thp-1 (Fig. 2). Androgen stimulated PSA-Luc activity in both CaP cells was reduced when in co-culture with equal number of THP-1 (Fig. 2A) or Saos-2 (Fig. 2B). A variety of cell lines, including MCF7 (Fig. 2C), ZR75 (Fig. 2D) and HepG2 (not shown), failed to change androgen-induced PSA-Luc reporter in both CaP cell types (Fig. 2). Reduced PSA-Luc reporter activity was observed with increasing number of Saos-2 cells in co-culture at different concentrations of androgens, either in combination with ZR75 to maintain equal the total number of cells in co-culture (Fig. 2E) or with Saos-2 alone (Fig. 2F). The maximum inhibitory

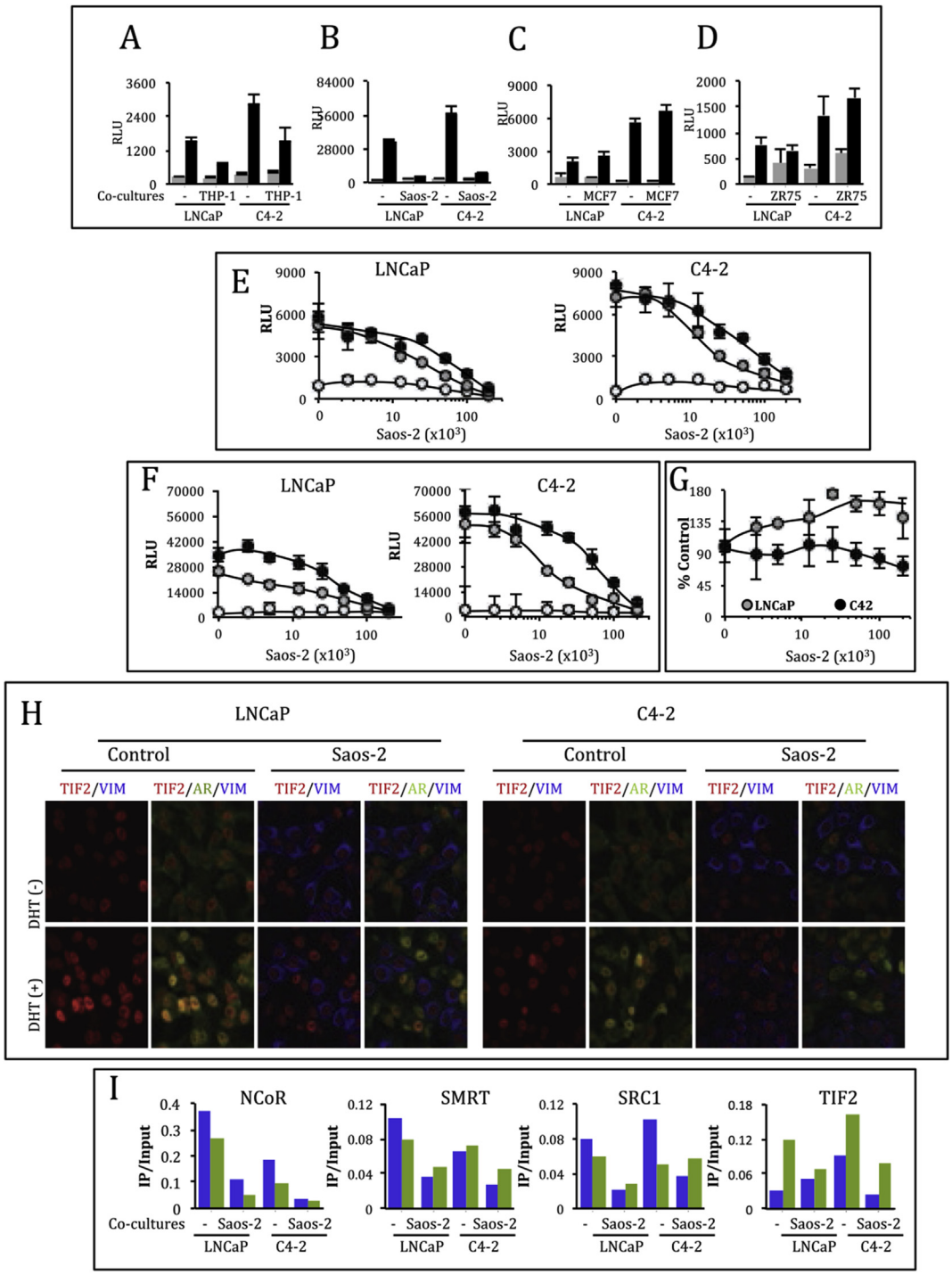

Fig. 2. Saos-2 and THP-1 cells in co-culture decreased assembly of coregulators on the AR complex in CaP cells. (A–D) Relative Luciferase activity of Ad-PSA-Luc reporter was determined in LNCaP and C4-2 in the absence (▩) and presence of 5 nM DHT (■), either alone (−) or in co-culture with THP-1 (A), Saos-2 (B), MCF7 (C), or ZR75 (D) cells. (E–F) The Ad-PSA-Luc reporter activity was determined in LNCaP and C4-2 with increasing number of Saos-2 ($0–2 \times 10^5$ cells), either in absence (○) or presence of 5 nM (●) or 50 nM (●) DHT. In (E), the total number of cells in co-culture was maintained constant by adding ZR75 to co-cultures. In (F), Ad-PSA-Luc reporter activity was determined in LNCaP and C4-2 with increasing number of Saos-2 alone ($0–2 \times 10^5$ cells). (G) Ad-PSA-Luc reporter activity in absence of DHT and increasing number of Saos-2 was plotted as changes in LNCaP (●) and C4-2 (●). (H) Representative images of immunofluorescence signal for TIF2 (red), AR (green) and Vimentin (blue) in LNCaP and C4-2 cultured alone (control) or with Saos-2, either in absence (−) or presence (+) of 10 nM DHT. The signal for nuclear localization was detected using Cy3-labeled secondary antibodies for TIF2 (red) and FITC-labeled for AR (green). The yellow signal in the nuclei of LNCaP and C4-2 indicated co-immunolocalization of AR and TIF2. Vimentin signal detection in blue (VIM) for Saos-2 was carried out as control. (I) Coregulator recruitment to PSA promoter in LNCaP and C4-2 was determined in the absence (▩) and presence (■) of 10 nM DHT using ChIP assays with antibodies against NCoR, SMRT, SRC-1 and TIF2 and specific primers for the PSA promoter. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

effect was observed with equal number of CaP and Saos-2 cells (Fig. 2E and F). Relative changes in PSA-Luc reporter activity with Saos-2 in androgen absence were minimal (Fig. 2G). Decreased AR-mediated transcription in CaP cells with Saos-2 was observed with Ad-MMTV-Luc reporter, either in direct co-culture or using the Transwell™ (data not shown). Decreased AR and TIF2 nuclear co-

localization using laser scanning and confocal microscopy supported decreased AR-mediated transactivation (Fig. 2H). AR-TIF2 co-localization was observed in LNCaP and C4-2 (shown in yellow) in the presence of androgens (Fig. 2H). The AR-TIF2 colocalization was reduced when LNCaP and C4-2 were incubated with Saos-2 (Fig. 2H, Saos-2). Similar decrease in co-immunolocalization was observed for AR-SRC-1 complex (data not shown). ChIP assays indicated that Saos-2 decreased recruitment of NCoR and SMRT; and SRC-1 and TIF2 to AR transcriptional complex (Fig. 2I).

3.3. Saos-2 and THP-1 change cell proliferation of LNCaP and C4-2 in cell-type specific manner

Mitotic index for LNCaP and C4-2 cultured alone or with Saos-2 or THP-1 was determined in the absence and presence of androgens (Fig. 3, Supplementary Fig. 1). LNCaP were active in proliferation in absence of androgens, with mitotic index of 16.1% (Fig. 3A). The pS10-H3 (16.5%) and *P*ropdium *I*odine *I*ncorporation (P.I.I.) indicated that LNCaP were in G0/G1 (80.1%), S (3.3%), and G2/M (16%). Co-culture with Saos-2 decreased the mitotic index to 4.9% and pS10-H3 to 5.7% (Fig. 3A). P.I.I. indicated increased G0/G1 to 90% and decreased G2/M to 4.8% (Fig. 3A). The Ki67marker in LNCaP with Saos-2 was decreased (data not shown). THP-1 increased the mitotic index to 37.7% and pS10-H3 to 36.8% in LNCaP (Fig. 3A). Consistently, P.I.I. was increased in S to 20% and G2/M to 33%, with decreased G0/G1 to 53.9% (Fig. 3A). Addition of androgens reverted the effect of Saos-2 (Fig. 3B). Mitotic index, pS10-H3, and cell cycle phases were restored to control levels observed in LNCaP cultured alone and with DHT (Fig. 3B). THP-1 continued to increase mitotic index and S-G2/M with DHT (Fig. 3B).

The C4-2 were active in proliferation in absence (Fig. 3C) and presence (Fig. 3D) of DHT, with similar mitotic index (Fig. 3). The pS10-H3 marker (41.3%) and P.I.I. indicated that C4-2 were in G0/G1 (49.7%), S (6.1%), and G2/M (42%) in androgen absence (Fig. 3C). Similar mitotic index and P.I.I. were observed for C4-2 with DHT, with increased S from 6.1% to 11.1% (Fig. 3C–D). Co-culture with either Saos-2 or THP-1 decreased the mitotic index and pS10-H3 in the absence (Fig 3C) and presence of DHT (Fig. 3D). The P.I.I. indicated that THP-1 and Saos-2 shifted the G0/G1, S and G2/M phases in C4-2 (Fig. 3C–D). Therefore, the effect of Saos-2 and THP-1 on CaP cells proliferation differed in absence and presence of androgens.

3.4. Saos-2 and THP-1 change global gene expression in CaP cells

Changes in mRNA expression signatures in LNCaP and C4-2 in co-culture with Saos-2 or THP-1 identified 103 genes involved in

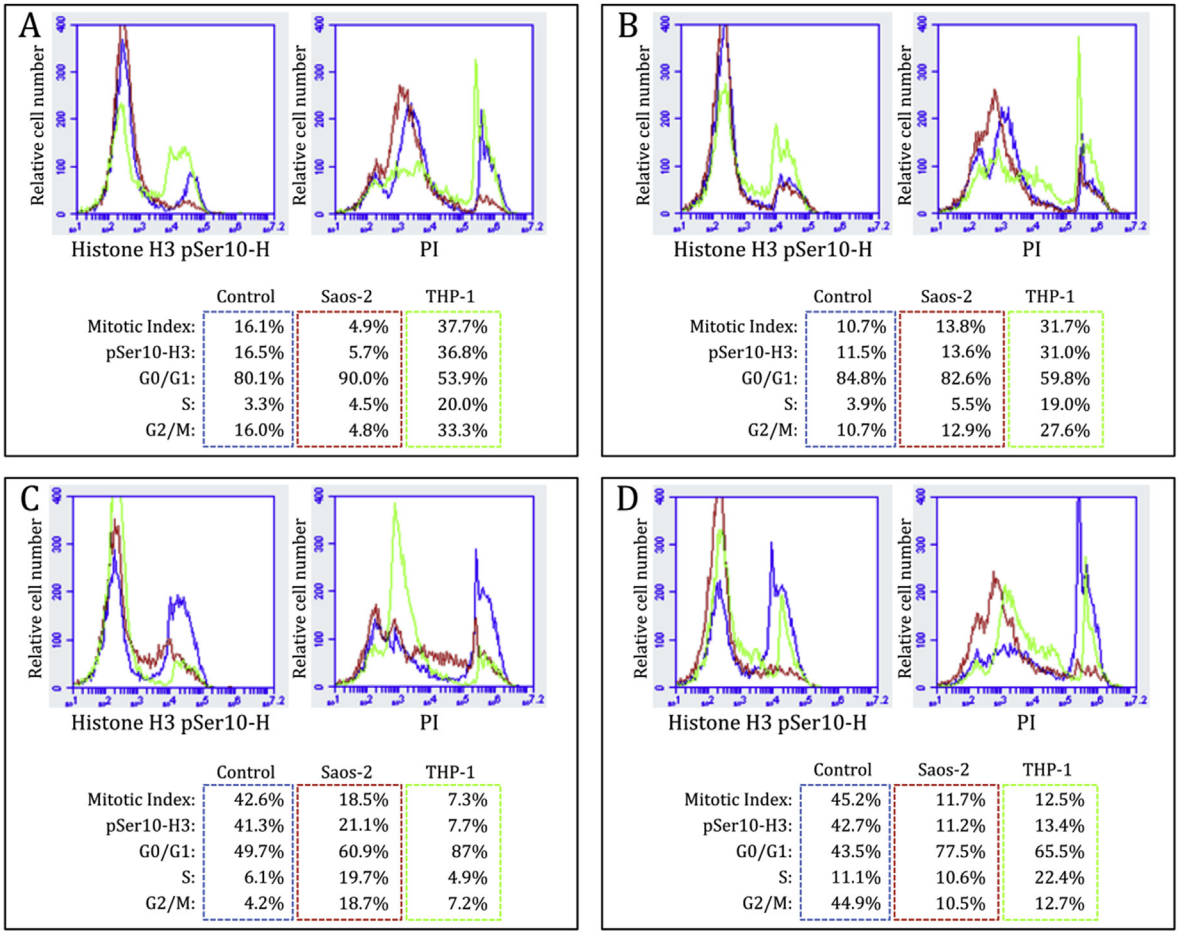

Fig. 3. Saos-2 and THP-1 cells change proliferation in CaP cells. Mitotic index flow cytometry assays using Histone H3-pSer10-H3 antibody (pS10-H3) in combination with propidium iodine incorporation (PI) coupled to FACS was used to determine the relative percent of CaP cells under proliferation in co-culture with Saos-2 or THP-1. The LNCaP (A,B) or C4-2 (C,D) cells were treated with 2% cdFBS containing media for 36 h and then cultured alone (■) or with Saos 2 (■) or THP-1 (■), either in the absence (A,C) or presence (B,D) of 10 nM DHT for 36 h using the Transwell™ system. The pS10-H3 was evaluated using FIT-C (FL1). PI incorporation into genomic DNA was used for cell cycle progression (FL2). Dot plot analysis (R17) was used to determine the mitotic cell index (Accuri C6, BD Biosciences). Relative number of cells positive for pS10-H3 and in different phases of the cell cycle (G0/G1, S or G2/M) are indicated as relative percent from a total of 2×10^4 events.

androgen regulation, and 83 genes involved in cell cycle. Differences in gene expression showing FC ≥ 1.9 were subjected to Venn diagrams (Fig. 4, Supplementary Table 1). In the absence of androgens, changes in 23 genes in LNCaP (13 up, 10 down) and 18 in C4-2 (14 up, 5 down) were identified as relevant in co-culture with Saos-2 or THP-1 (Fig. 4A). Only one gene encoding Interleukin 6 (IL6) was decreased in common with |−3.8| for LNCaP and |−6.3| for C4-2 (Fig. 4A). Two genes critical for steroid hormones synthesis, hydroxy-delta-5-steroid dehydrogenase (HSD11B1) and hydroxysteroid (11-beta) dehydrogenase 1 (HSD3B1), were upregulated (Fig. 4A). The mediator coactivator complex (MED4) that modulates RNA pol II driven genes was identified in common with |−2.6| for LNCaP and |4.0| for C4-2 (Fig. 4A). Five different genes, including AR, ETS family of transcription factors (SPDEF), HEY1 corepressor and THRAP5 coactivator were upregulated in LNCaP and downregulated in C4-2 in the presence of bone stroma-derived cells (Fig. 4A).

In the presence of androgens, changes in 26 genes in LNCaP (18 up, 8 down) and 27 in C4-2 (22 up, 5 down) were identified as relevant in co-culture with Saos-2 or THP-1 (Fig. 4B). Only one gene encoding the orphan Testicular Receptor (NR2C1) was decreased in common, with |−3.9| for LNCaP and |−5.8| for C4-2 (Fig. 4B). Two genes relevant for cell division, including cell division cyclin 25B (CDC25) and pro-apoptotic and transcriptional repressor death-domain associated protein (DAXX), were upregulated. The p21 (Cdc42/Rac)-activated kinase 6 (PAK6) involved in AR trafficking and glutathione peroxidase (GPX5), were upregulated in both CaP cells (Fig. 4B). Corepressor NCoR1 was differentially expressed with |3.4| for LNCaP and |−3.5| for C4-2 (Fig. 4B). Several genes encoding coregulators were increased, including SUMO1, NOCA2, NCOA5, NCOA7, LCORL and HEYL, among others (Supplementary Table 1).

Twenty-four genes involved in cell cycle were identified in CaP cells in absence of androgens (Supplementary Table 1). The |−2.6| for LNCaP and |4.0| for C4-2 in the transcription factor TFDP2 that increases expression of cell cycle regulated genes, and the |−5.0| for cyclin-dependent kinase 6 relevant for G1/S transition in LNCaP, suggest a role for decreased proliferation of LNCaP with Saos-2 and increased with THP-1. The FC in |−11.0| for cyclin T1 (CCNT1) required for RNA pol II elongation suggest a role for decreased expression of androgen-regulated genes. Thirty-nine genes involved in cell cycle with FC FC > |1.9| were identified in the presence of androgens (Supplementary Table 1).

4. Discussion

Coactivators form high-order complexes that enhance transcription activity mediated by nuclear receptors due to chromatin remodeling structure for better access to promoters in target genes [6]. A distinct multi-subunit coactivator complex, the activator-recruited cofactor (ARC) complex, is involved in both coactivation and corepression of genes [6]. The ARC complex is devoid of TF-IID, SRC-1 and p300/CBP and suggests the presence of several preformed coactivator complexes that can be used in sequential or combinatorial manner to regulate transcription [6]. Pre-formed multi-subunit coactivator complexes are models for changing AR specificity between different cell types. Increased AR activity in C4-2 was the result of increasing AR and SRC-1/TIF2 recruitment, as well as decreased NCoR expression. Therefore, androgen-resistant CaP may contain unique preformed multi-subunit coactivator complexes that lack corepressor(s) and provide a mechanism for AR to work as a transcription factor in the low circulating testicular androgens.

Genome-wide expression studies identified several genes that are downregulated in advanced CaP due to attenuated AR signaling [16]. Consistently, the AR activity in both CaP cell types was decreased with Saos-2 and THP-1 due to decreased overexpressed SRC-1/TIF2 and decrease of the already reduced SMRT/NCoR

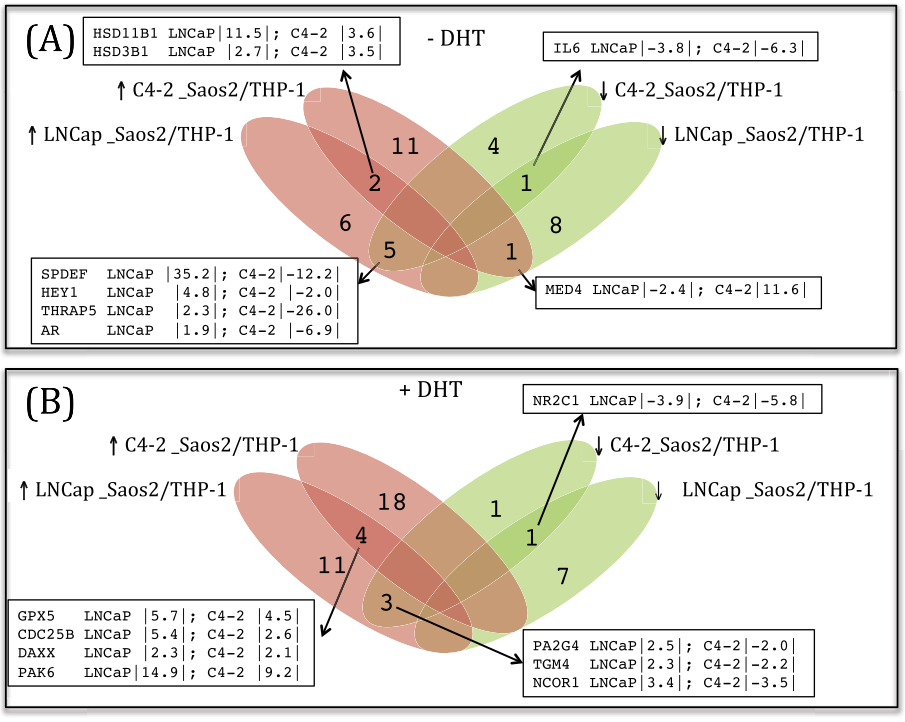

Fig. 4. Venn diagrams. The diagrams represent a comparison in the number of genes with increased (Red) or decreased (Green) FC ≥ |1.9| mRNA expression in LNCaP and C4-2 in co-cultures with Saos-2 and THP-1 with potential expression signatures in androgen regulation (A–B), either in absence (−DHT) or presence of 10 nM androgens (+DHT). The specific fold changes for each gene involved in androgen regulation and cell cycle is described in Supplementary Table 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

recruitment to AR complex, in presence of androgens. Therefore, CaP metastasis may acquire a new AR that is released to an apparently unbound state in the bone stroma. The finding that LNCaP and C4-2 express different sets of coregulators when in absence and presence of bone stroma-derived cells suggests that several new preformed coactivator complexes may be used by AR within the RNA pol II initiation complex, in either a sequential or combinatorial manner, to alter target gene expressions involved in androgen hypersensitivity or metastasis reactivation.

The effect of bone stroma cells on the cell cycle regulation is complex [17], and may involve post-translational modifications of cell cycle regulators. Important genes encoding cell cycle regulators, including transcription factor Dp-2 and anaphase-promoting complex subunit 2, are reduced in the presence of Saos-2 and may account for decreased proliferation in both CaP cell types. Despite the cell proliferation decrease in C4-2 cells in short-term co-culture with both Saos-2 and THP-1 in absence and presence of androgens, cell cycle regulator expressions are diverse. On the other hand, increased cell cycle regulators expression, including recombinase RAD51 and cyclin B2, may account for the effect of THP-1 in increasing proliferation in androgen-sensitive LNCAP cells. Decreased cyclin T1 (CCNT1) expression required for RNA pol II activity may reduce androgen-regulated gene expression. However, several cell cycle regulators were upregulated in CaP cells in co-culture with bone stroma-derived cells.

Two genes critical for steroid hormone synthesis, including hydroxy-delta-5-steroid dehydrogenase (HSD11B1) and hydroxysteroid (11-beta) dehydrogenase 1 (HSD3B1), were significantly upregulated in both cells types co-cultured with Saos-2 and THP-1. This suggests that bone-derived stroma cells may induce intracrine synthesis of androgens after short-term interaction. This synthesis is relevant to intracrine and paracrine action within the bone stroma cell microenvironment and may be facilitated by GnRH agonist/antagonist bimodal treatment, which in spite of decreasing circulating androgens, increases intracellular DHT levels to above 3.4 nM [18].

Newly synthesized AR coregulators in CaP cells at the bone niche is presumably due to interactions with paracrine grow factors derived from bone marrow stroma cells, supported by an environment of age and androgen deprivation therapies that decrease circulating androgens. The altered complex of coregulators and transcription factors that compete for access to regulate and integrate diverse signaling pathways in CaP cells represents changes in either individual components or in the summation of all proteins recruited to the transcriptional complex. Thus, reactivation of new androgen-regulated signaling pathways in bone metastasis tumor cells may promote progression to androgen hypersensitivity, castration resistance and lethal phenotype.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.10.009.

Transparency document

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