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Androgen receptor- and PIAS1-regulated gene programs in molecular apocrine breast cancer cells

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ABSTRACT

We have analyzed androgen receptor (AR) chromatin binding sites (ARBs) and androgen-regulated transcriptome in estrogen receptor negative molecular apocrine breast cancer cells. These analyses revealed that 42% of ARBs and 39% androgen-regulated transcripts in MDA-MB453 cells have counterparts in VCaP prostate cancer cells. Pathway analyses showed a similar enrichment of molecular and cellular functions among AR targets in both breast and prostate cancer cells, with cellular growth and proliferation being among the most enriched functions. Silencing of the coregulator SUMO ligase PIAS1 in MDA-MB453 cells influenced AR function in a target-selective fashion. An anti-apoptotic effect of the silencing suggests involvement of the PIAS1 in the regulation of cell death and survival pathways. In sum, apocrine breast cancer and prostate cancer cells share a core AR cistrome and target gene signature linked to cancer cell growth, and PIAS1 plays a similar coregulatory role for AR in both cancer cell types.

1. Introduction

The majority of breast cancers and breast cancer subtypes express the androgen receptor (AR) in addition to the estrogen receptor α (ERα) (Park et al., 2012). Depending on the breast cancer subtype, AR and androgen signaling can have either tumor suppressive or oncogenic role on breast cancer growth. A complex interplay between ERα and AR seems to explain the opposite androgen responses in different subtypes of breast cancers (Cochrane et al., 2014; De Amicis et al., 2010; Ni et al., 2011). Gene profiling studies have identified among ERα-breast cancers a molecular apocrine subtype that expresses high levels of AR together with a gene expression pattern resembling that of ERα+ luminal breast cancer. The subtype is highly aggressive with a poor prognosis (Lehmann-Che et al., 2013). MDA-MB453 cell line is ERα−/AR+/HER2+ and thus considered a model for the molecular apocrine subtype of breast cancer (Doane et al., 2006). Growth of MDA-MB453 cells is similar to that of AR+ prostate cancer cells, in that it is promoted by androgens and inhibited by AR antagonists or AR silencing (Doane et al., 2006; Robinson et al., 2011). In ERα+ breast cancers, AR expression is associated with a lower grade disease and a better prognosis, whereas in ERα− breast cancers, AR appears to be capable of mediating proliferation and thus acting an oncogenic driver (Dent et al., 2007; Tsang et al., 2014). As AR-targeting therapies are available and effective in prostate cancers, the AR could offer a therapeutic target also in ERα−/AR+ breast tumors.

Genome-wide analyses have revealed that, by and large, AR regulates transcription via AR-binding sites (ARBs) in distal enhancers instead of binding to promoter regions (Pihlajamaa et al., 2015; Tang et al., 2011). In addition to androgen response elements (AREs) in the ARBs, the region of AR chromatin occupancy encompasses cis-elements for other transcription factors (TFs), such as pioneer TFs GATA-binding protein 2 (GATA2) and forkhead box A1 (FOXA1), E twenty-six or E26 transformation-specific (ETS) TFs and homeodomain protein HOXB13 (Carroll et al., 2005; Sahu et al., 2011; Wu et al., 2014). Regulation of transcription by AR

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also involves numerous AR-interacting coregulator proteins. These coregulators include SWI/SNF chromatin-remodeling complexes and steroid receptor coactivators and corepressors that harbor or recruit enzymatic activities that "write" or "erase" histone marks, such as acetylation or methylation marks (Heemers and Tindall, 2007; Rosenfeld et al., 2006).

Tissue-specific differences in the expression and activity of coregulators and those of other TFs are thought to significantly contribute to the cell- and target gene-selective regulation by the AR (Burd et al., 2006; Heemers et al., 2010; Linja et al., 2004). On the other hand, dysregulated expression of the coregulators, such as protein inhibitor of activated STAT1 (PIAS1) (Rytinki et al., 2009), may be involved in AR-linked diseases, including prostate cancer (Hoefer et al., 2012). PIAS1 is capable of promoting protein SUMOylation as a SUMO ligase, and interestingly, it functions in prostate cancer cells as an AR target gene selective coregulator (Toropainen et al., 2015). Overexpression of PIAS1 has also been observed in breast cancer samples and associated with increased cell growth (Liu et al., 2014). Moreover, SUMOylation and PIAS1 have shown to play a key role in the transcriptional maintenance of basal breast cancer phenotype (Bogachek et al., 2014). Evidence for a role of the PIAS1 in suppression of TGF-β-induced activation of matrix metalloproteinase MMP2 in breast cancer cells has been also reported (Dadakhujaev et al., 2014). However, there is no genome-wide information of the role that PIAS1 plays in the regulation of AR target genes in breast cancer cells.

In this work, we mapped genome-wide chromatin binding sites (cistrome) of AR in the MDA-MB453 cells by using chromatin immunoprecipitation deep sequencing (ChIP-seq) and analyzed the cistrome in relation to the androgen-regulated transcriptome. We also studied the role of PIAS1 in the regulation of AR's target gene programs and cistrome in MDA-MB453 cells and compared these data with the AR transcriptome and cistrome in VCaP prostate cancer cells. Our results show that the MDA-MB453 and the VCaP cells share a core AR cistrome and target gene signature and that PIAS1 plays a similar coregulatory role both in apocrine breast cancer and in prostate cancer cells.

2. Materials and methods

2.1. Cell culture

Human breast cancer cell line MDA-MB453 was obtained from the American Type Culture Collection (Manassas, VA, USA). The cell line was maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Invitrogen) supplemented with 10% (v/v) fetal bovine serum (FBS), 25 U/ml penicillin and 25 μg/ml streptomycin in a 5% CO_2 atmosphere at 37 °C. R1881 (methyltrienolone) was from Steraloids Inc. (Newport, RI).

2.2. RNAi

Cells were transfected with small interfering RNA (siRNA) oligomers (ON-TARGETplus SMARTpool and non-targeting pool (siNON) control, Dharmacon, Lafayette, CO, USA) using Trans-IT-siQUEST reagent (Mirus Bio Corp.) according to the manufacturer's instructions. The cells were incubated for 72 h and then exposed to vehicle or 10 nM R1881 for the next 16 h (gene expression profiling) or for 2 h (ChIP-seq).

2.3. Isolation of RNA, real-time quantitative PCR (RT-qPCR) and microarray analysis

Total RNA of biological triplicates was extracted using TriPure (Roche Diagnostics GmbH, Mannheim, Germany) and converted to cDNA using Transcriptor First Strand cDNA synthesis Kit (Roche) according to the manufacturer's instructions. cDNA was used as a template in RT-qPCR, which was carried out using LightCycler 480 SYBR Green I Master (Roche) and LightCycler 480 System (Roche) and with specific primers listed in (Supplementary Table 1). Analyzed *GAPDH* mRNA levels were used to normalize the amounts of total RNA between the samples. Fold changes were calculated using the formula amplification efficiency-(ΔΔCt), where ΔΔCt is ΔCt(R1881)−ΔCt(EtOH), ΔCt is Ct(gene X) −Ct(*GAPDH*) and Ct is the cycle at which the threshold is crossed. Illumina HumanHT-12 v4 Expression BeadChip (San Diego, CA, USA) analyses were carried out at the Finnish Microarray and Sequencing Centre (Turku, Finland) using protocols recommended by the manufacturer. The data were analyzed and visualized as described (Sutinen et al., 2014a). The genes with adjusted p-value of <0.01 and fold change ≥1.5 and ≤0.7 were selected as significantly changed transcripts. Heat maps were generated by using heat map.2 in the R package gplots. Ingenuity Pathway Analysis (IPA) was used to identify biological processes differently enriched between siNON and siPIAS1 cells. First, a core analysis was performed with two distinct lists (androgen-regulated genes in siNON or siPIAS1 cells). The results were then compared to identify any distinct biological processes that were differentially regulated.

2.4. Chromatin immunoprecipitation coupled to high-throughput sequencing (ChIP-seq)

Antibodies used for ChIP assays (and immunoblotting) were rabbit polyclonal anti-AR (Karvonen et al., 1997), normal rabbit IgG (sc-2027, Santa Cruz) and rabbit monoclonal anti-PIAS3+PIAS1+PIAS2 (ab77231, Abcam). Immunoblot analyses from PIAS1-silenced cells clearly showed that the latter antibody specifically recognizes PIAS1 in the MDA-MB453 cells (cf. Fig. 2A). ChIP experiments were performed as described previously (Paakinaho et al., 2014). Briefly, cells were exposed to vehicle or 10 nM R1881 for 2 h prior to fixing in 1% (v/v) formaldehyde and harvesting for immunoprecipitation with antibodies. ChIP samples were processed according to Illumina's instructions. DNA libraries were sequenced using Solexa/Illumina 1.5 System in the Biomedicum Functional Genomics Unit (Helsinki, Finland). Raw read quality check, preprocessing of reads (36-bp trimming) and Bowtie alignment were performed as described (Sutinen et al., 2014a). Samples were scaled against input background for both AR and PIAS1 ChIP-seq samples and the binding sites, getDifferentialPeaks, motifs analysis (known motif enrichment analysis) and visualization of the data were performed as described (Toropainen et al., 2015). Peak identification was based on their occurrence in both replicates with 3-fold enrichment over background and tag count ≥6. The AR and PIAS1 binding sites were associated to nearby androgen-regulated genes (±100 kbp from gene TSS) with Anduril (Engine 1.2.18) as described (Toropainen et al., 2015).

2.5. Apoptosis assay

Cells were transfected with siNON or siPIAS1 on 6-well plates. After 72 h, the cells were trypsinized and split onto 96-well plates (15 000 cells/well). After 24 h, the cells were exposed to either vehicle or 10 nM R1881 for the next 72 h. The amount of apoptotic cells was measured using the Cell Death Detection ELISAPLUS kit according to the manufacturer's instructions.

2.6. Immunoblotting

Western blots were processed as previously described (Rytinki et al., 2011) and analyzed by immunoblotting with the same

antibodies used for ChIP-seq or anti-tubulin (sc-5286) antibody. The appropriate secondary antibody was from Invitrogen and chemiluminescence detection reagents from Pierce (Rockford, IL).

2.7. External datasets

VCaP bead array data (GSE30316), AR and PIAS1 ChIP-seq data (GSE56086) (Toropainen et al., 2015), MDA-MB453 AR cistrome (mapped to hg19, E-MTAB-986, Robinson et al., 2011) and MCF-7 ER cistrome (GSE68356, Mohammed et al., 2015) were used in comparison analyses.

2.8. Accession numbers

ChIP-seq and gene expression microarray data have been deposited to the NCBI Gene Expression Omnibus database (Edgar et al., 2002) (http://www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO Series accession number GSE70163.

3. Results and discussion

3.1. PIAS1 regulates AR target gene program in molecular apocrine breast cancer cells

Quantitative immunoblotting indicated that MDA-MB453 (from now on MB453) breast cancer cells contain ~3-fold more PIAS1 protein than VCaP cells, whereas the amount of AR protein in VCaP prostate cancer cells is ~4-fold higher than that in MB453 cells (Fig. 1). To decipher the genomic androgen signaling and function of PIAS1 in MB453 cells, PIAS1 was depleted from MB453 cells using transfection with PIAS1-specific siRNAs (siPIAS1), and cells transfected with non-targeting siRNAs (siNON, control) were used as a control. Silencing of PIAS1 was confirmed by immunoblotting with anti-PIAS1 antibody (Fig. 2A). Gene expression profiling of cells exposed to synthetic non-aromatizable androgen (R1881) or vehicle revealed that more genes were regulated by androgen in PIAS1-depleted cells than in control siNON cells (290 versus 153 genes) (Fig. 2B) (listed in Supplementary file 1). PIAS1 depletion rendered a new group of genes (160) more sensitive to androgen regulation. The group contained both androgen-induced (64) and -repressed (96) genes (Fig. 2B). The number of genes affected (fold change $\geq$1.5 and $\leq$0.7) by PIAS1 depletion in the absence of androgen (13 genes, Supplementary file S1) was much smaller than in the presence of androgen, indicating that PIAS1 is relatively selective to AR target genes in MB453 cells. These genome-wide expression data indicate that PIAS1 modulates, either co-activates or co-represses, in a gene-selective manner a number of AR target genes in MB453 cells, which is reminiscent of the co-regulatory role of PIAS1 on AR target gene expression in VCaP prostate cancer cells (Toropainen et al., 2015).

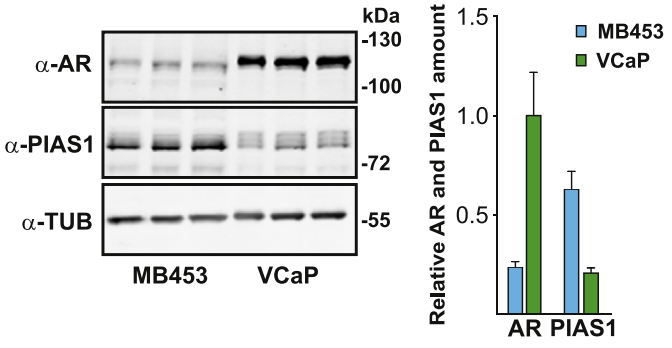

Fig. 1. AR and PIAS1 protein levels in MB453 and VCaP cells. Cell lysates were immunoblotted with anti-AR, anti-PIAS1 and anti-tubulin (TUB, used as a loading control) antibodies (left panel). Three independent cell lysate samples were quantified by the Odyssey Infrared system, and the results are presented as mean ± SD in relation to tubulin in the same samples (right panel).

3.2. Molecular and cellular functions of androgen-regulated genes in MB453 cells

Next, the androgen-regulated genes of control (153) and siPIAS1-depleted (290) cells were subjected to Ingenuity Pathway Analysis (IPA) to identify enriched molecular and cellular functions among them (Fig. 2C). In both control and PIAS1-depleted cells, cell death and survival, cellular growth and proliferation, cellular development, cell signaling, gene expression and cellular movement were the top six significantly enriched molecular and cellular functions. Since these analyses suggested involvement of PIAS1 in the cell death and survival pathway, we examined apoptosis levels in control and PIAS1-depleted MB453 cells in the presence or absence of androgen. Interestingly, PIAS1 depletion lead to a significantly lower level of apoptosis, and androgen exposure further reduced the amount of apoptotic cells (Fig. 2D). This result suggests that PIAS1 is capable of promoting apoptosis in molecular apocrine breast cancer cells.

We recently analyzed AR target gene profiles in VCaP prostate cancer cells under the same androgen exposure conditions by using the same analysis platform (Toropainen et al., 2015), permitting comparison of the MB453 data to those from VCaP cells. Thirty-nine % (59/153) of the androgen-regulated genes in control and 33% (95/290) in PIAS1-depleted MB453 cells overlapped with those in control and in PIAS1-depleted VCaP cells, respectively (Toropainen et al., 2015) (Supplementary Fig. S1). Androgen had a similar up- or down-regulating effect on 42 of the AR target genes shared between the MB453 and VCaP cells (Fig. 2E). Based on IPA, most (30/42) of these genes are linked to cancer. Comparison of the androgen-regulated genes in MB453 cells to those in VCaP cells revealed that cell death and survival, cellular growth and proliferation, cellular development, and cellular movement are among the top six significantly enriched IPA molecular and cellular functions also in VCaP cells, despite the prostate cancer cells possess nearly 9-fold more androgen-regulated genes than MB453 cells (Toropainen et al., 2015), (Supplementary Fig. S2). However, there were also interesting differences in the IPA functions between the cell lines; for example, cellular response to therapeutics, amino acid metabolism and drug (and steroid) metabolism showed a significant ($p < 0.05$) enrichment only in MB453 cells (Supplementary Fig. S2). Notably, the UDP glucuronosyltransferase 2B (UGT2B) family members *UGT2B7*, *UGT2B10*, *UGT2B11* and *UGT2B28* which are involved in the catabolism of estrogens and androgens (Levesque et al., 2001) are robustly up-regulated by androgen in MB453, but not in VCaP cells (Supplementary Fig. S3). Interestingly, silencing of PIAS1 conferred strong androgen up-regulation on *UGT2B11* and *UGT2B28* in VCaP cells and enhanced their androgen induction also in MB453 cells.

3.3. Genome-wide chromatin occupancy of the AR in molecular apocrine breast cancer cells

To complement the target gene expression analyses, we mapped the AR cistrome in MB453 cells using ChIP-seq. The sequence reads of two biological replicates (showing a good concordance, as judged by tag density comparisons, Supplementary Fig. S4A) were aligned to human reference genome and significantly enriched peaks were called using HOMER software. The ChIP-seq analyses revealed 17 396 ARBs in both replicates. Comparison with a recently reported MB453 cell AR cistrome (Robinson et al., 2011) with our data

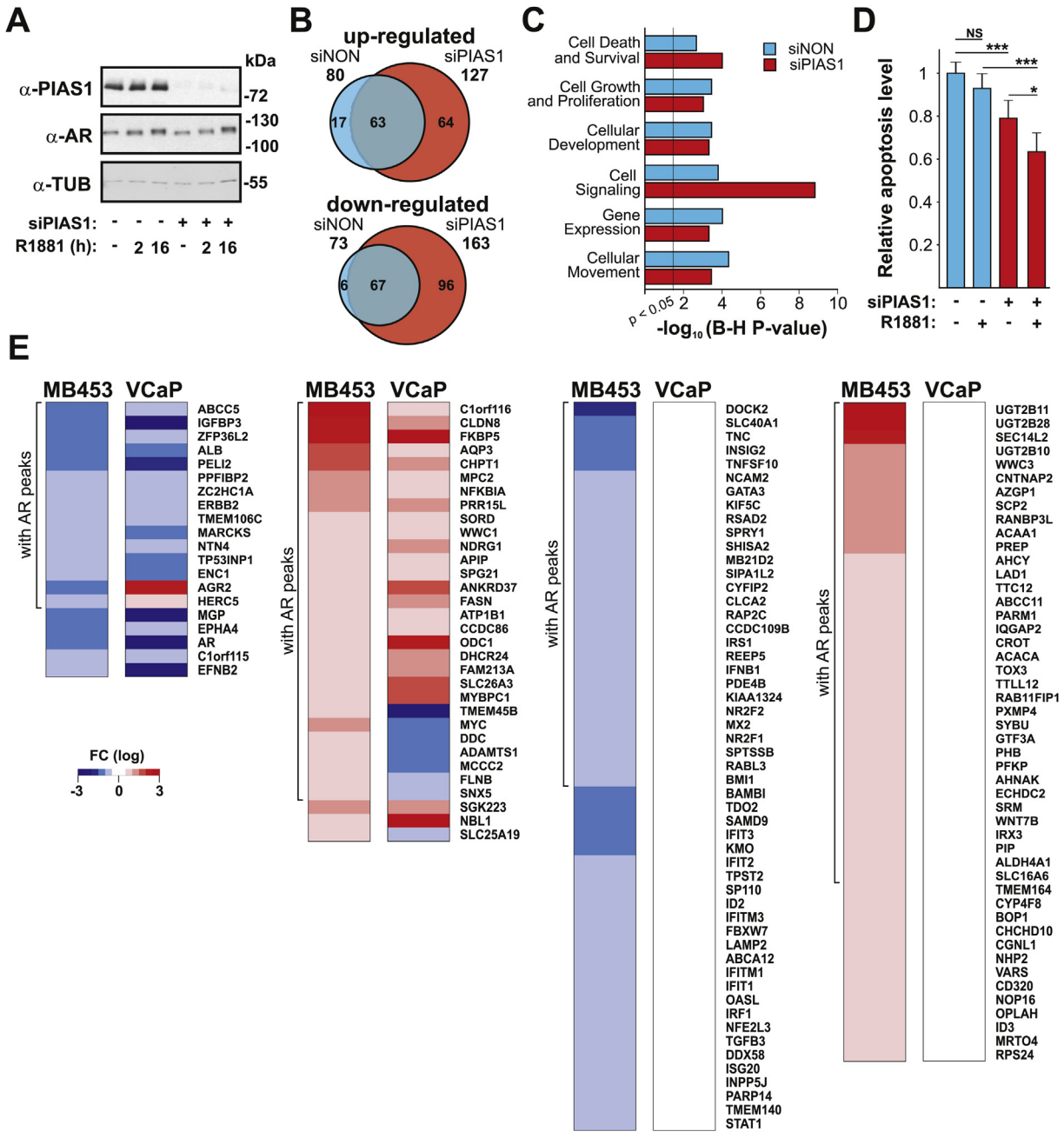

Fig. 2. PIAS1 depletion perturbs with the expression of androgen-regulated genes in MB453 breast cancer cells. The cells were transfected with control siRNA (siNON) or PIAS1 siRNA (siPIAS1). (A) Immunoblot analysis of PIAS1 and AR levels in PIAS1-depleted cells. MB453 cells exposed to vehicle (−) or R1881 (10 nM) for 2 h or 16 h were immunoprecipitated with anti-PIAS1, anti-AR and anti-tubulin antibodies. (B) For expression BeadChip array, cells were exposed to R1881 (10 nM) or vehicle for 16 h with biological triplicates. The genes with adjusted fold change of ≥1.5 and ≤ 0.7 and p-value of <0.01 were considered as androgen-regulated genes in siNON and siPIAS1 cells. Venn diagrams showing androgen up- and down-regulated genes in siNON (blue) and siPIAS1 cells (red). (C) Comparative analysis of the top significantly enriched molecular and cellular functions of androgen-regulated transcripts in siNON or siPIAS1 cells as revealed by Ingenuity pathway analysis. Benjamini–Hochberg (B–H) multiple testing correlation was used for enrichment analysis. (D) Effect of PIAS1 depletion on the apoptosis of MB453 cells. Cells were exposed to vehicle or R1881 for 72 h. Relative amount of apoptotic cells were measured using Cell Death Detection ELISAPlus kit (SD, n = 3). Statistically significant differences between siNON and siPIAS1 cells are indicated (***P < 0.001 and *P < 0.05; Student's t-test). (E) Heat maps showing androgen-regulated genes in MB453 in comparison to their androgen regulation in VCaP cells. The color key from blue to white to red indicates the logarithmic fold change (FC (log)) of transcripts down-regulated (blue) or up-regulated (red) by androgen. Genes associated with AR ChIP-seq peaks (±100 kb from the gene) and shared between MB453 and VCaP cells in a window of ±100 kb are identified by "with AR peaks". (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)

showed a good (63%) overlap (Supplementary Fig. S4B). We also compared our MB453 AR cistrome with our VCaP cell AR cistrome of 41 469 ARBs identified using the same analysis platform and settings: 42% of ARBs in MB453 cells were the same as those in VCaP (Toropainen et al., 2015) cells (Fig. 3A), with the shared ARBs possessing the highest tag density numbers in both cell lines (Fig. 3A the lowest panel). Thus, the ARBs with the most robust AR occupancy – the highest affinity AR-binding sites – appear to be shared between molecular apocrine breast and prostate cancer cell lines. Others have shown that the AR cistrome in MB453 cells is in

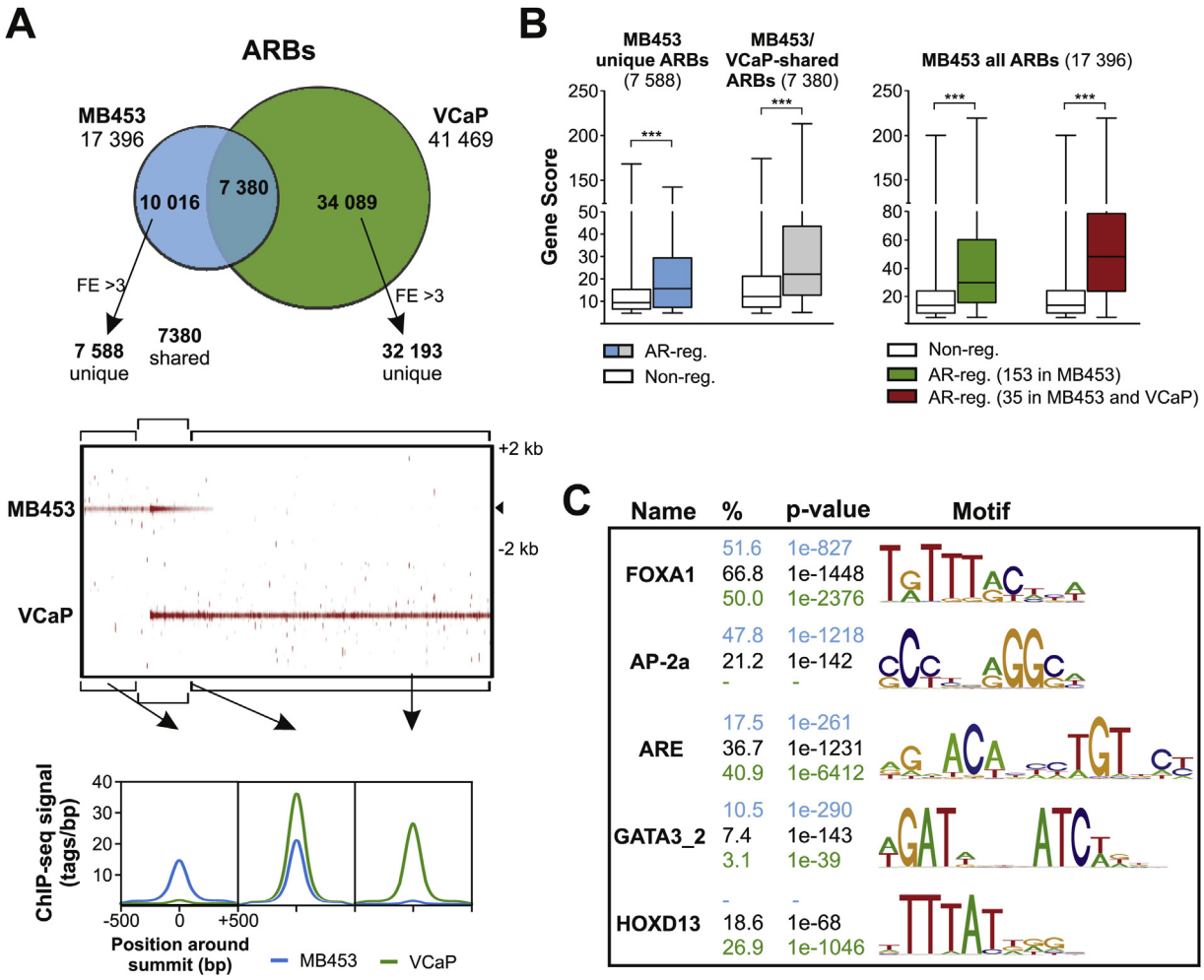

Fig. 3. AR-binding sites (ARBs) in MB453 breast cancer cells in comparison to those in VCaP prostate cancer cells. AR ChIP-seq analysis was carried out for both cell lines under the same experimental conditions (10 nM R1881 for 2 h). (A) Venn diagram (upper panel) shows the overlap of ARBs and the heat map (middle panel) depicts tag densities for ARBs identified in MB453 and VCaP cells (Toropainen et al., 2015; GSE56086). Bottom panel shows comparison of average AR tag counts in ±500 bp from the centers of ARBs in MB453-unique, MB453/VCaP-shared and VCaP-unique sites. (B) Gene scores (>5) for the genes linked to nearby peaks were calculated by taking into consideration the peak depth normalized tags, the distance (±100 kb) and the peak counts. (Left panel) Gene scores for MB453 cell unique ARBs and VCaP cell-shared ARBs were calculated to all androgen-regulated genes in MB453 cells. (Right panel) Gene scores for all MB453 ARBs were calculated to all androgen-regulated genes or to the AR genes shared between MB453 and VCaP cells. The significances were calculated between androgen-regulated and non-regulated gene scores by one-way ANOVA (Kruskal–Wallis test) (***P < 0.001). (C) Comparison of the top five enriched DNA-binding motifs within the ARBs in MB453 and VCaP cells. Percentage of peaks with the motif and p-value of enrichment are color-coded (MB453 unique, blue; MB453/VCaP-shared, black; VCaP unique, green). (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)

fact more similar to the ERα cistrome in MCF-7 breast cancer cells (51% overlap) than the AR cistrome in LNCaP prostate cancer cells (29% overlap), with the strongest AR-binding sites again being the ones shared between the cell lines (Robinson et al., 2011). Our AR cistrome in MB453 cells showed 29% overlap with ERα cistrome in MCF-7 cells (Mohammed et al., 2015), whereas the AR cistrome in VCaP cells displayed only 10% overlap with the MCF-7 cell ERα cistrome. Fig. 4A shows snapshots of *IGFBP3*, *C1orf116*, *CLDN8*, and *FKBP5* as examples of loci that are AR-occupied and androgen-regulated in both MB453 and VCaP cells. In addition to the MB453- and VCaP-shared ARBs, the latter three loci showed cell line unique ARBs. *OPLAH* and *CSRP2* in turn represent examples of loci that have ARBs and are androgen-regulated merely in MB453 cells or in VCaP cells, respectively (Fig. 4B and C).

The majority (>70%) of the androgen-regulated genes in MB453 control cells were associated with at least one ARB within ±100 kb of their TSS. To further analyze association of genes to the nearby ARBs, a gene score that takes into account the ARB peak signal strength (normalized tags), distance between the ARB and TSS and number of associated ARBs was calculated for each gene. Both the MB453-preferred and the MB453- and VCaP-shared ARBs showed a significantly stronger association with androgen-regulated genes than with non-regulated genes (Fig. 3B). The shared ARBs showed higher gene scores than the MB453-preferred ones. Moreover, the 35 AR target genes with at least one ARB within ±100 kb from the gene and a similar androgen regulation in both MB453 and VCaP cells (cf. Fig. 2E) displayed even higher gene scores. Of note, each gene in the latter group associated with several ARBs, with the median number of ARBs being 5.5 and 6.6 for androgen up-regulated genes and 3 and 6 for androgen down-regulated genes in MB453 and VCaP cells, respectively.

Motif analysis to identify transcription factor-binding motifs enriched within all MB453 cell ARBs revealed highly significant enrichment for FOXA1, AP-2α, ARE and GATA motifs (Fig. 3C). The AP-2α motif was significantly enriched within the breast cancer cell-preferred ARBs, but not within the prostate cell cancer-preferred ARBs, and in comparison to the latter ARBs, ARE motifs were interestingly underrepresented among the breast cancer cell-

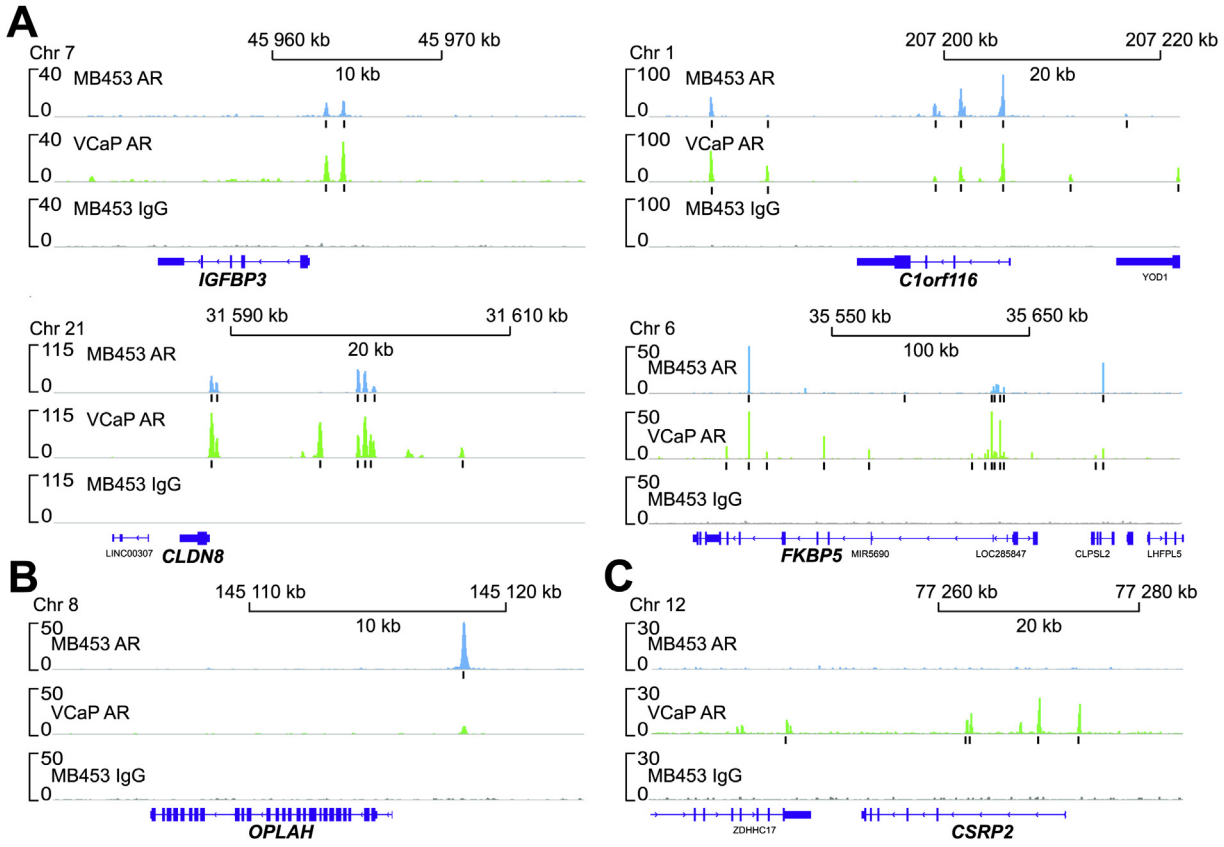

Fig. 4. ChIP-seq track examples of AR-binding events in AR target loci in MB453 and in VCaP cells. (A) The occupancy of AR in *IGFBP3*, *C1orf116*, *CLDN8*, and *FKBP5* loci shared between MB453 and VCaP cells. (B and C) The occupancy of AR in *OPLAH* locus specific for MB453 cells and *CSRP2* locus specific for VCaP cells, respectively. IgG, control antibody track in MB453 cells.

preferred ARBs. HOXD13 motif, in turn, was entirely absent within the MB453-preferred ARBs. In ERα+ MCF-7 breast cancer cells, AP-2 motifs have been found to be enriched within ERα-binding sites. AP-2γ has been shown to bind to these motifs in estrogen-dependent fashion, and depletion of AP-2γ (TFAP2C) has been reported to impair ERα-mediated long-range chromatin interactions (Tan et al., 2011). Moreover, AP-2γ collaborated with the pioneer factor FOXA1 in MCF-7 cells. Since the occupancy of AP-2 motifs influenced chromatin binding of ERα (Tan et al., 2011), it is plausible that AP-2 motif-binding TFs play a similar role with AR in molecular apocrine breast cancer cells. Enrichment of FOXA1 motifs within ARBs in MB453 cells is in agreement with the FOXA1's role in pioneering androgen-regulated gene programs in ERα-breast cancer cells (Ni et al., 2011; Robinson et al., 2011). Additional collaboration of the AR with the AP-2 motif-binding TFs is likely to be one of the mechanisms targeting AR to breast cancer cell specific ARBs in MB453 cells. Interestingly, AP-2α motifs and the AP-2α cistrome have recently been shown to be substantially overlapping with tissue-specific ARBs in another androgen target tissue, mouse epididymis (Pihlajamaa et al., 2014).

3.4. PIAS1 occupies a subset of AR chromatin targets in MB453 cells

Since PIAS1 is capable of interacting with AR on prostate cancer cell chromatin (Toropainen et al., 2015), we performed ChIP-seq with PIAS1 antibody in the presence of R1881 in MB453 cells and analyzed sequence reads of two biological replicates (Supplementary Fig. S5). The genome-wide analysis revealed that the binding of PIAS1 is enriched at 640 genomic regions that overlapped almost completely (>95%) with the ARBs in MB453 cells (Fig. 5A). However, the PIAS1 cistrome showed only a <4% overlap with the AR cistrome, which is clearly smaller than that (~10%) in VCaP cells (Toropainen et al., 2015). The MB453 PIAS1 cistrome overlapped somewhat more with the AR cistrome in VCaP cells than with the ERα cistrome in MCF-7 cells (66% vs. 57%, respectively) (Mohammed et al., 2015). The motif analysis of PIAS1-binding sites revealed essentially the same motifs as in the ARBs, with the FOXA1 motif being the most enriched cis-element and found at 70% of the PIAS1-binding sites (Supplementary Fig. S6). The latter result is likely to reflect the interaction of PIAS1 with FOXA1, as was seen in VCaP cells (Toropainen et al., 2015). Similarly to the situation in VCaP cells (Toropainen et al., 2015), the ARBs showing the strongest AR-binding events were the ones overlapping with the PIAS1-binding sites (Fig. 5A lower panel and B). Additionally, more than one third of the MB453 PIAS1-binding sites – the ones corresponding to the strongest PIAS1 peaks – were shared with VCaP cells. Fig. 5C shows snapshots of *MYBPC1*, *ATAD2* and *SMPDL3A* loci as examples of co-occupancy of AR and PIAS1 in MB453 cells.

We also examined whether PIAS1 influences AR occupancy on chromatin. MB453 cells were transfected with control or siPIAS1 and exposed to R1881. Analysis of tag densities of AR-binding events showed that only 651 new ARBs and 3 lost ARBs were unique to PIAS1 depletion (Fig. 5D). As PIAS1 depletion had only relatively small effects on AR chromatin occupancy, but clear effects on AR target gene expression, the mechanism by which PIAS1 regulates AR target genes in molecular apocrine breast cancer cells may involve promotion of FOXA1 SUMOylation. This is likely to take

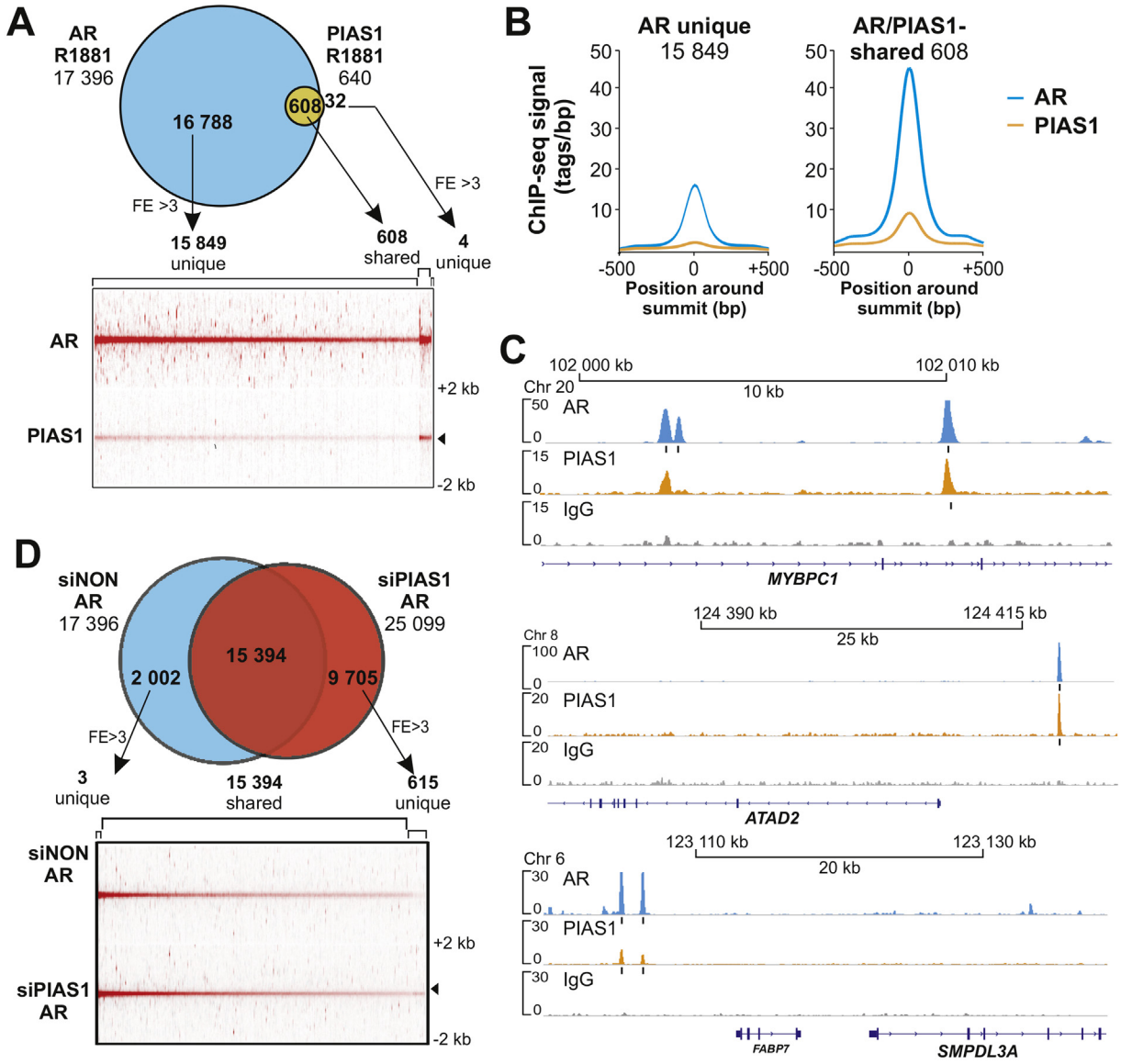

Fig. 5. PIAS1-binding sites on MB453 cell chromatin and the effect of PIAS1 depletion on AR chromatin occupancy. (A) Venn diagram shows the overlap of AR- and PIAS1-binding sites in presence of androgen (upper panel) and the heat map depicts AR and PIAS1 tag densities for AR unique, AR/PIAS1-shared and PIAS1 unique regions in a ±2 kb window (lower panel). (B) Comparison of average tag density counts in a ±500 bp window from the centers of AR unique and AR/PIAS1-shared binding sites. (C) ChIP-seq track examples of AR- and PIAS1-binding loci in MB453 cells. The occupancy of AR (blue) and PIAS1 (orange) is shown in the presence of R1881. (D) AR ChIP-seq analysis of MB453 cells depleted from PIAS1. Venn diagram showing the overlap of AR cistromes in siNON and siPIAS1 cells exposed to R1881 for 2 h (upper panel). Heat map showing AR tag densities for siNON and siPIAS1 sites in a ±2 kb window (lower panel). (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)

place off-chromatin, but consequently regulate the activity of FOXA1 with the AR on-chromatin (Sutinen et al., 2014b). PIAS1 may also regulate AR target gene expression in breast cancer cells by regulating transcriptional activity of AP-2 motif-binding factors (Bogachek et al., 2014). Since PIAS1 has additionally been reported to regulate breast tumorigenesis by contributing to epigenetic histone marks and chromatin structure (Liu et al., 2014), another intriguing possibility is that PIAS1 cooperates with coregulator proteins responsible for modifying chromatin structure and histone marks of AR target genes. In sum, these data imply that PIAS1 has potentially multifaceted roles in the regulation of gene programs relevant to breast cancer cell growth and signaling.

Disclosure statement

The authors have nothing to disclose.

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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.mce.2015.07.024.

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