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14. ABSTRACT

Androgen signaling via its receptor, AR, remains the key therapeutic target in prostate cancer. Despite advances in treatment, disease ultimately recurs. Our goal is to inhibit AR target genes that drive cancer growth but retain expression of those for normal cell differentiation. Our hypothesis is that these sets of genes differ in androgen response elements (AREs), with genes driving proliferation relying on consensus inverted repeats (cARE) and genes promoting differentiation relying on selective direct repeats or half-sites (sAREs). Compounds interacting with any AR domain, or interacting molecules, may alter AR conformation to affect response element recognition. To identify such compounds, we developed a high-throughput screen for differential AR action with transfected fluorescent reporters driven by multimerized cAREs or sAREs. Over 10,000 compounds from several libraries, including 2,000 FDA-approved drugs, were tested in multiple screens. The two best hits were confirmed in rescreens and validated for differential effects on AR activity *in vitro* and *in vivo*. In particular, these compounds differentially affect AR-dependent gene expression in LNCaP cells. We are currently pursuing the basis for a difference in AR binding to DNA and within chromatin, and will test differential effects in mouse cancer models.

15. SUBJECT TERMS

androgen receptor, prostate cancer, antiandrogens, high-throughput screen, differential promoter activation, selective response elements

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Table of Contents

Page

1. Introduction……………………………………………………………. 4

2. Keywords…………………………………………………………. 4

3. Accomplishments………..…………………………………………... 4

4. Impact…………………………...…………………………………… 11

5. Changes/Problems...….………………………………………………… 12

6. Products…………………………………….……….….……………. 12

7. Participants & Other Collaborating Organizations…………… 12

8. Special Reporting Requirements……………………………………… n/a

9. Appendices………………………………………………………… n/a

Selective AR Modulators that Distinguish Proliferative from Differentiative Gene Promoters

1. INTRODUCTION: Prostate cancer (PCa) initially depends on androgens acting via the androgen receptor (AR), and thus blocking steroid synthesis and AR function slows disease. Yet even with potent new drugs, resistance arises and AR activity recurs. An innovative approach to deter resistance is to identify selective AR modulators (SARMs) that prevent expression of genes promoting cancer growth but permit expression of genes for differentiation. Promoters of such genes may differ in androgen response elements (AREs) and cofactor binding sites. Supporting this hypothesis, genes involved in differentiation appear to rely on selective AREs (sAREs), characterized as direct repeats of the 6 bp half-site, unlike the consensus AREs (cAREs) that are inverted repeats shared with other steroid receptors. To identify SARMs that elicit differential gene expression, we developed a high-throughput promoter-dependent compound screen, aided by the University of Michigan Center for Chemical Genomics. Differential AR activation in transfected cells was assessed using fluorescent reporter genes driven by multimerized cAREs or sAREs. This strategy targets AR regardless of hormone presence or receptor variation (e.g., splice forms), and may identify compounds contacting any AR domain or interacting with components of the AR signaling pathway. This versatile assay can identify compounds applicable to nearly all clinically relevant scenarios. A pilot screen of 2500 compounds identified a small set strongly suppressing cARE but not sARE reporters, and some with opposite preference. One characterized hit provides proof-of-concept by differentially regulating AR target genes *in vivo*.

2. KEYWORDS: androgen receptor, prostate cancer, antiandrogens, high-throughput screen, differential promoter activation, selective response elements

3. ACCOMPLISHMENTS: The major goals stated in the Statement of Work were:

Task 1. Validate pilot screen hits as modulators of AR gene regulation and define mechanisms.

Task 2. Perform an optimized and larger screen for additional diverse SARMS.

Task 3. Test antitumor efficacy of selective modulators *in vivo*.

We have made significant progress and describe below first, the screen (*Task 2*), and then validation of hits from this larger screen as well as the promising one from the earlier pilot screen (*Task 1*).

Compound Screening. In a pilot screen of 2500 FDA-approved compounds and drugs used in clinical trials, a small set of compounds that strongly suppressed the consensus (cARE) but not selective (sARE) fluorescent reporters were identified. The drug doxorubicin (dox) was a lead hit. A larger primary screen was developed that enhanced efficiency by utilizing a 3-step approach rather than simultaneous screening for selective AR modulators:

Step 1. Compounds were screened for ability to inhibit cARE-driven transcription.

Step 2. Hits were retested for dose-dependent inhibition of cARE-driven transcription.

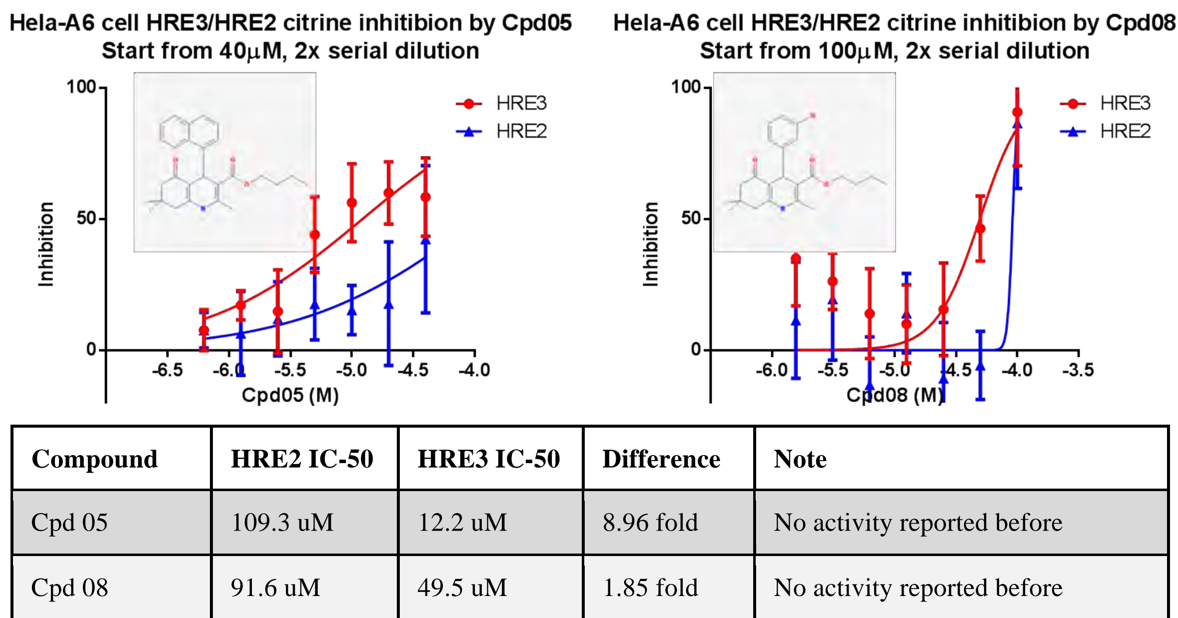
Step 3. Hits from Step 2 were tested for dose-dependent inhibition of sARE-driven transcription

This strategy essentially halved the time and reagents needed for screening. The protocol was optimized using reverse transfection of citrine fluorescent reporters into HeLa cells stably expressing AR (HeLa-A6). Screening was carried out in saturating levels of the synthetic androgen R1881 (100 nM) to ensure maximal separation of activation from inhibition. The procedure is summarized as follows:

Day 1: Premixed complex of Effectene transfection reagent and reporters are plated in 384-well plates, before adding cells suspended in growth media with 10% fetal bovine serum (FBS).
Day 2: 20 h post-transfection, cells are washed with 1x PBS and growth media with 2.5% charcoal-stripped serum added to starve the cells. After 8 h, R1881 +/- compound is added.
Day 3: Reporter activity is measured 22 h after hormone treatment.

Compound activity was noted as inhibition of R1881-induced fluorescence. Screening cutoffs were set at greater than 70% signal inhibition and less than 50% cell toxicity (determined by Promega's Cell Titer Glow assay). 7612 compounds were screened from the following libraries: Prestwick (1280 compounds), LOPAC (1280), MS2400 (960), Biofocus natural products (892) and 3200 compounds from the ChemDiv 100K libraries. Of 124 primary hits (1.62% hit rate) (*Step 1*), 109 showed dose dependent inhibition of cARE-citrine (*Step 2*). 15 of the 109 compounds did not inhibit sARE-citrine, indicating selectivity (*Step 3*), for a screen hit rate of 0.2% (15/7612 compounds). One hit is a topoisomerase I inhibitor, similar to doxorubicin, a topoisomerase II inhibitor, which was a lead hit in the pilot. Both compounds intercalate into DNA, further suggesting a structural difference between cARE and sARE sequences that could influence AR or coregulator binding and modulate AR signaling.

Hit confirmation. 18 of the 109 anti-cARE compounds were from the ChemDiv 100K library and 7 were selective in failing to inhibit sARE-citrine. A confirmation assay with fresh powders of the 18 compounds was performed in HeLa-A6 cells transfected with cARE- or sARE reporters (see Fig. 1). For optimal results, the cells were transfected as a pool and then re-plated in individual wells to ensure consistency among wells and to economize on reagent. In addition, to minimize cell disruption caused by the automatic microplate washer / dispenser, a programmable hand-held multichannel pipet was used. These modifications improved the consistency of the reporter signal and enhanced its strength by 2.8 fold.

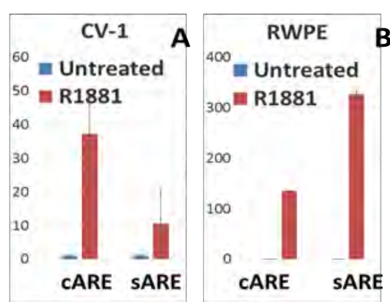


Compound	HRE2 IC-50	HRE3 IC-50	Difference	Note
Cpd 05	109.3 uM	12.2 uM	8.96 fold	No activity reported before
Cpd 08	91.6 uM	49.5 uM	1.85 fold	No activity reported before

Fig. 1. Cpd05 demonstrated clear differential effect of suppressing cARE but not sARE in promoter-fluorescent reporter assay. HeLa-A6 cells were transfected with cARE (HRE3) or sARE (HRE2) driven citrine fluorescent reporter and treated with R1881 and compounds.

All 18 compounds were dissolved in DMSO and 7 concentrations (in quadruplicate) used to generate inhibition curves. As shown in Fig. 1, Cpd05 from the larger screen showed differential inhibition of cARE vs. sARE, indicating efficacy of the screen. The calculated IC-50 for cpd05 shows selectivity of about 8.96 fold between cARE and sARE. Cpd08 has a similar chemical structure to cpd05, and also demonstrated selectivity between cARE and sARE but of only 1.85 fold. In the PubChem database, no reports of activity of cpd05 were found while cpd08 was reported inactive in 7 assays. Thus Cpd05 is the lead hit from the larger screen, and the first report of activity for this compound.

Validation of high-throughput screen hits in transient transfection assays. Dox and Cpd05 were next compared for differential suppression of luciferase as well as fluorescent reporters in cotransfection assays in CV-1 fibroblasts and RWPE-1 normal prostate epithelial cells. Both cell lines transfect well and generate robust luciferase signals but differ in hormonal response. Fold induction is greater and less variable in RWPE cells than in CV-1 cells. Also, sARE-reporters are more responsive than cARE in RPWE cells, while the opposite is seen in CV-1 cells (Fig. 2A, B). Such promoter preferences likely reflect differences in host cell factors. Prostate cancer cell lines such as LNCaP, VCaP and LAPC-4 were not used for this assay because of their low transfection efficiencies.



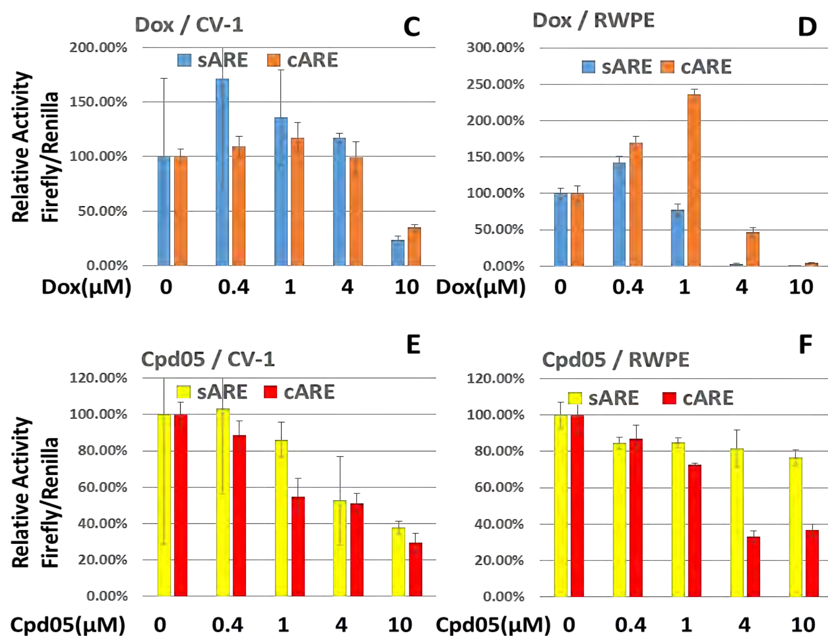


Fig. 2. Dox and Cpd05 suppress cARE and sARE differently in CV-1 and RWPE cells. cARE or sARE driven reporters were co-transfected with AR into CV-1 and RWPE cells for Dual Luciferase Assays. Transfected cells were starved with 2.5% CSS and then treated with R1881 (1 nM) and compounds or DMSO.

As shown in Fig. 2 (E, F), Cpd05 repressed cARE-driven reporters to a greater extent than sARE reporters in both CV-1 and RWPE cells. Dox repressed cARE reporters more than sARE reporters only in CV-1 cells, consistent with previous data. These observations validate the screen results, and indicate that dox and Cpd05 may regulate by different mechanisms, with dox more cell type specific than Cpd05. The data also indicate that dox can stimulate sARE- luciferase at lower concentrations than Cpd05. Dox, by unknown mechanisms, increases expression of tumor suppressors p21 and p53; data here indicates dox may also enhance expression of AR targets.

Effect of compounds on endogenous gene expression. To determine whether the differential effects of dox and Cpd05 occur on natural promoters, we examined their effects on a panel of 7 AR target genes (AQ3, KLK1, NKX3, TMPRSS2, FKBP5, SGK1, P21) in the prostate cancer cell line LNCaP. Cells were starved in media with 2.5% CSS and then treated with R1881 and compounds. Fig. 3 demonstrates that dox and cpd05 both effectively repressed R1881 induction of FKBP5, KLK1, NKX3 and TMPRSS2 in LNCaP cells. Further, dox and Cpd05 both stimulated P21, which is only modestly androgen-induced, and AQ3, which is repressed by R1881 in LNCaP cells. Dox and Cpd05 differed in their effect on SGK1 expression, which is stimulated by dox but repressed by Cpd05.

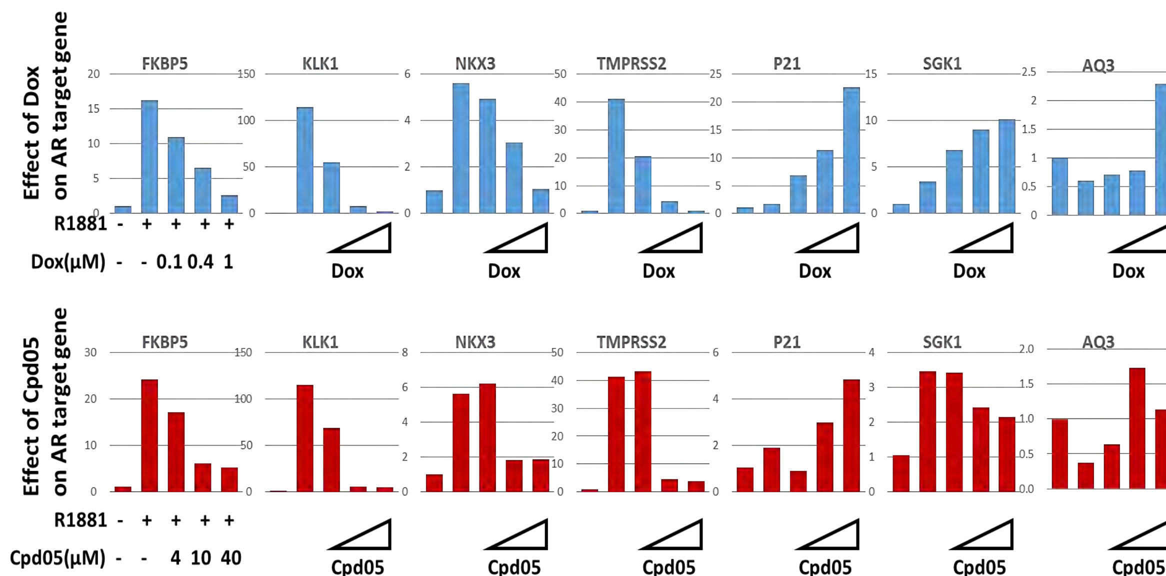


Fig. 3. Dox and Cpd05 differentially regulate AR target gene expression in LNCaP cells. LNCaP cells were starved in media with 2.5% CSS, treated with R1881 and compounds for 24 h, and RNA extracted and assayed by RT (real time) q-PCR. Data were normalized to GAPDH.

FKBP5, KLK1, NKX3 and TMPRSS2 are well known AR target genes and their repression by dox and Cpd05 was expected. For genes involved in AR-mediated proliferation or differentiation, both dox and Cpd05 showed anti-proliferative activity by inducing P21, a cell cycle regulator that functions as a tumor suppressor (1). Both dox and Cpd05 relieved the R1881 repression of AQ3, which is associated with differentiation. However, dox stimulated while Cpd05 repressed expression of SGK-1, which is one of the few genes known to be driven by sAREs (2). In normal prostate RWPE cells stably transduced with AR (RWPE-AR), dox and Cpd05 showed similar but more modest trends (data not shown), suggesting such effects may be greater in malignant than benign prostate cells. Thus both dox and Cpd05 stimulated differentiation-related genes and repressed proliferation-related genes, with similar but not identical patterns, again suggesting they may have different regulatory mechanisms.

Effect of compounds on cell growth. Since both dox and Cpd05 elicited differential endogenous gene regulation, we tested the effect of dox on AR-driven cell proliferation. Prostate cancer cell lines LNCaP and LAPC-4 were cultured in media with 10% CSS and treated with 10 nM R1881 and low concentrations of dox for 5 days. At low dose, dox selectively blocked AR-induced proliferation (Fig. 4). At higher doses, dox blocked cell proliferation regardless of R1881 (data not shown).

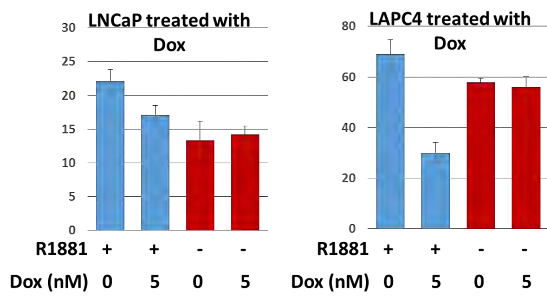


Fig. 4. Dox inhibits AR-induced prostate cancer cell growth. LNCaP and LAPC4 cells were starved in media with 10% CSS and then treated with dox for 5 days. Growth was assessed by Trypan blue exclusion counting. Blue – with androgen; red – without androgen.

These data indicate a delicate balance for dox regulation of AR signaling. We hypothesize that at lower concentration, blocking of AR-ARE interaction plays a role in inhibiting proliferation but activating differentiation; at high concentration, dox-induced DNA damage response (DDR) and apoptosis more universally inhibits proliferation. There is growing interest in the interaction between AR and DDR pathways as targets for prostate cancer therapy (3, 4), supporting the need for further investigation into the mechanism of AR pathway interactions in the validation of these lead compounds.

Effect of dox on AR/DNA binding. We suspect that either dox differentially binds cARE and sARE DNA sequences, or that dox binding to these sequences differentially affects subsequent AR binding. To test this, Electrophoretic Mobility Shift Assays (EMSA) with AR, ^{32}P-labeled cARE or sARE probes and differing concentrations of dox were performed. The probes were 29 bp in length, with the cARE inverted repeat (TGTTCT half-sites separated by a 3 bp linker) or sARE direct repeat flanked by 7 bp extra nucleotides on both 5' and 3' ends. 1 ng ^{32}P-labeled probe with 5 µg nuclear extract and varying amounts of dox was incubated on ice and then applied to EMSA gels.

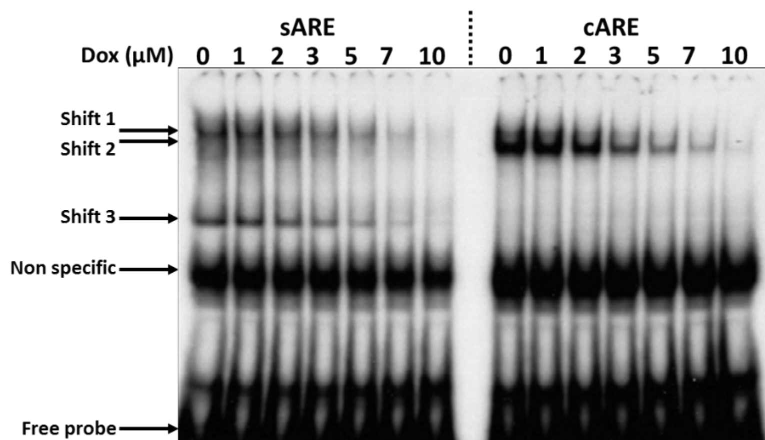


Fig. 5. Dox inhibits AR-cARE, AR-sARE binding in VCaP cells. 5 µg total nuclear extract from VCaP cells was incubated with 1 ng ^{32}P labeled sARE or cARE probes and dox on ice before EMSA.

The assay was first carried out in prostate cancer VCaP cells that have very high AR expression. EMSA revealed several shifted bands (Fig. 5) above the free probe. The non-specific band was not sensitive to dox treatment. The Shift 1, Shift 2 and Shift 3 bands varied with dox concentration. Shift 1 appeared with both sARE and cAREs; Shift 2 just below Shift 1 appeared only with cARE; Shift 3 appeared only with sARE.

To clarify the EMSA results, we performed this assay with nuclear extracts from AR-transfected HEK-293T cells, and observed similar shift patterns (Fig. 6A). Shift 1 depended on AR presence (compare to untransfected HEK-293T extracts in the 2 right lanes in each half of Panel A), varied with dox concentration and appeared in both sARE and cARE lanes, strongly supporting that Shift 1 was the product of AR-sARE or AR-cARE specific binding. Shift 1 bands in sARE lanes were weaker than in cARE lanes, reflecting the 4-fold weaker AR-sARE binding affinity than AR-cARE binding affinity (5). As in

VCaP cells, Shift 2 and Shift 3 were also dox dose dependent but appeared regardless of AR presence, implying they were due to binding of other proteins. To further confirm this, we added AR antibody N-20 in EMSA assays with AR-transfected HEK-293T cells (Fig. 6B). Shift 1 bands were now “super-shifted”, i.e. ran slower than Shift 1. The shifts also appeared stronger due to the known effect of antibody stabilizing the AR/ARE complex. In contrast, Shift 2 and Shift 3 were not affected by N-20 antibody. This confirmed that Shift 1 represented specific AR binding, whereas Shift 2 and 3 bands reflected binding of non-AR proteins. It is intriguing that these non-AR shifts differ between sARE and cARE and are affected by dox. The data also support the notion that dox is disrupted AR/ARE binding by interacting with the ARE sequence and not directly with AR.

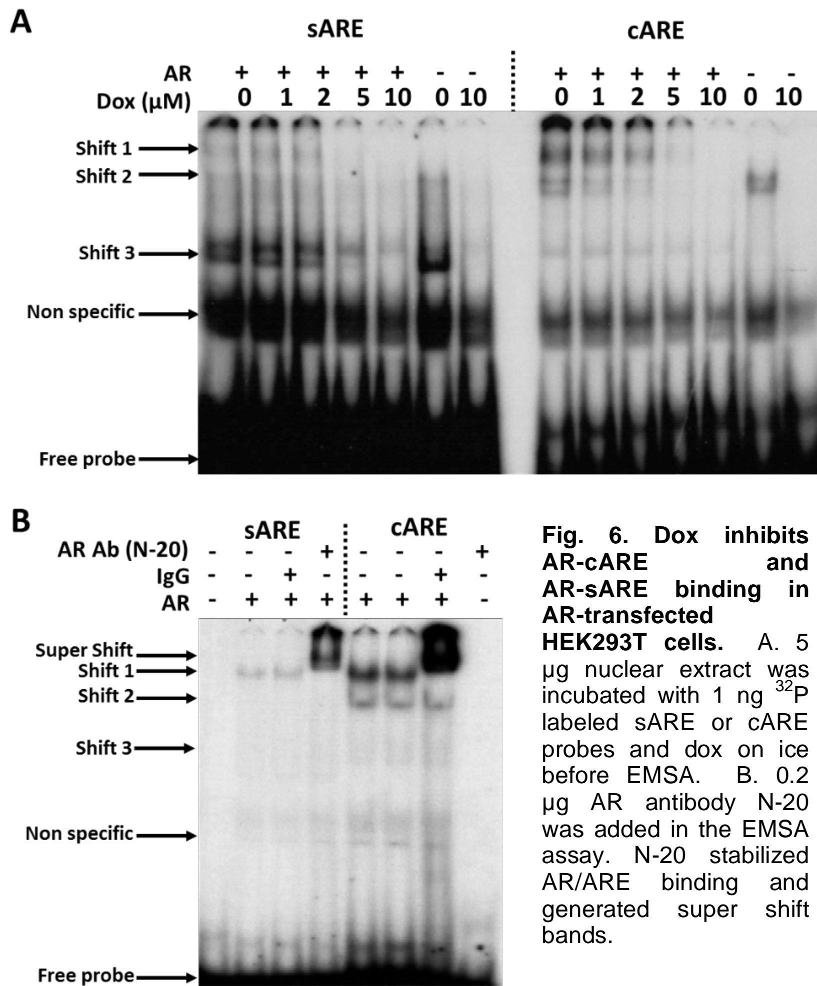


Fig. 6. Dox inhibits AR-cARE and AR-sARE binding in AR-transfected HEK293T cells. A. 5 μg nuclear extract was incubated with 1 ng ^{32}P labeled sARE or cARE probes and dox on ice before EMSA. B. 0.2 μg AR antibody N-20 was added in the EMSA assay. N-20 stabilized AR/ARE binding and generated super shift bands.

We next quantified the effect of dox on AR binding to the two response elements in repeated EMSA assays in HEK-293T cells (Fig. 7). These assays did not use AR antibody to avoid artefacts from complex stabilization. Reaction times were limited to 20 min on ice to minimize nonspecific binding, since time courses showed that both specific binding and effects of dox occurred very fast (data not shown). As shown below in Fig. 7, AR-cARE binding is more sensitive to dox treatment than AR-sARE binding. In accord with the gene expression data, this effect is evident at low dox concentration, while higher dox inhibited binding to both elements similarly. Relative binding was analyzed by by Image J quantification (Fig. 7B). Similar results were obtained in Hela-A6 cells (data not shown), thus confirming the selective effect of dox on AR binding in two different cell lines.

Since dox binds to DNA and not directly to AR, it may be that there is a differential binding affinity of dox to cARE and sARE sequences that produces the subtly distinct outcome. Alternatively, intercalation of dox into DNA may have sequence-dependent structural effects on the helix and thus have distinct affects dependent on whether AR is binding to the inverted repeat of a cARE or a direct repeat (or

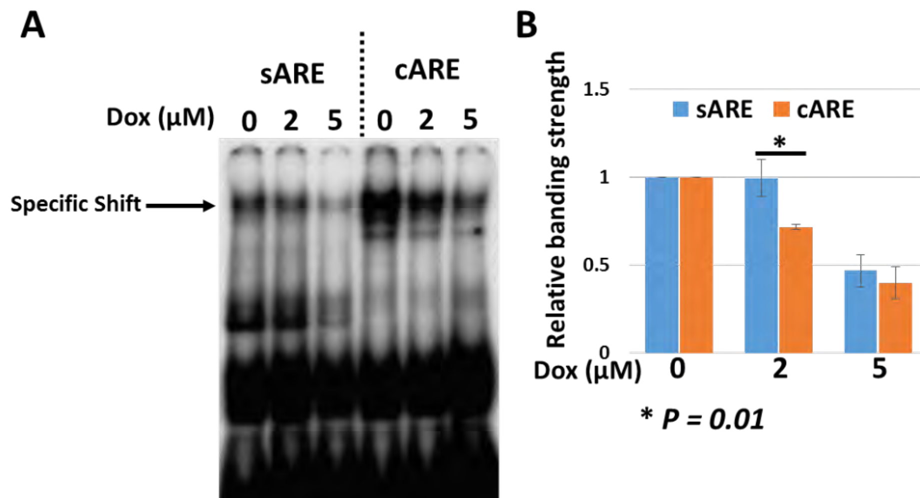


Fig. 7. AR-cARE binding is more sensitive to dox treatment at low concentration than AR-sARE binding in HEK 293T cells. 5 µg nuclear extract was incubated with 1 ng ^{32}P-labeled sARE or cARE probes and dox on ice before EMSA.

half-site) as in a sARE. In low concentration, dox may bind to a cARE element more strongly than to sAREs, or perturb the element structure to more greatly affect AR binding. These subtle differences in differential AR/ARE binding become magnified at the level of gene expression. This ultimately may redirect cell fate towards differentiation rather than proliferation.

Thus in sum, our data has validated the screening results for two compounds and has confirmed the original project hypothesis in an *in vitro* system. Our immediate future goals remain to further validate the selective effects of these antiandrogens in an endogenous chromosome context, particularly for known androgen-responsive genes, and in *in vivo* studies in preclinical mouse xenograft models. Long-term goals include determining whether the selective effects of dox can be enhanced by combination with other drugs, or treatments such as radiation.

KEY RESEARCH ACCOMPLISHMENTS:

- Performed larger scale high-throughput screen of over 7500 compounds to identify selective AR antagonists. Re-testing and confirmation identified Cpd05 as a lead compound of the primary screen.
- Validated that Cpd05 and dox, identified in pilot assays, suppressed cARE but not sARE activity in luciferase-reporter assays.
- Demonstrated that dox and Cpd05 affected the transcription of AR target genes differently, with a more pronounced effect observed in the prostate cancer cell line LNCaP than in normal prostate epithelial RWPE cells.
- Showed that dox inhibited AR-dependent growth of the prostate cancer cell lines LNCaP and LAPC4.
- EMSA assays were used to study the basis of the differential effect of dox on AR binding to cAREs vs. sAREs, and indicate that either dox has a higher binding affinity to cARE sequences or causes different structural perturbations affecting the affinity of AR for cARE vs sARE sequences.

REFERENCES

1. Warfel NA, El-Deiry WS. p21WAF1 and tumourigenesis: 20 years after. *Curr Opin Oncol.* 2013;25(1):52-58.
2. Schauwaers K, De Gendt K, Saunders PT, Atanassova N, Haelens A, Callewaert L, Moehren U, Swinnen JV, Verhoeven G, Verrijdt G, Claessens F. Loss of androgen receptor binding to selective androgen response elements causes a reproductive phenotype in a knockin mouse model. Proc Natl Acad Sci U S A. 2007;104(12):4961-4966.
3. Goodwin JF, Schiewer MJ, Dean JL, Schrecengost RS, de Leeuw R, Han S, Ma T, Den RB, Dicker AP, Feng FY, Knudsen KE. A hormone-DNA repair circuit governs the response to genotoxic insult. *Cancer Discov.* 2013;3(11):1254-1271.
4. Polkinghorn WR, Parker JS, Lee MX, Kass EM, Spratt DE, Iaquinta PJ, Arora VK, Yen WF, Cai L, Zheng D, Carver BS, Chen Y, Watson PA, Shah NP, Fujisawa S, Goglia AG, Gopalan A, Hieronymus H, Wongvipat J, Scardino PT, Zelefsky MJ, Jasin M, Chaudhuri J, Powell SN, Sawyers CL. Androgen receptor signaling regulates DNA repair in prostate cancers. *Cancer Discov.* 2013;3(11):1245-1253.
5. Schoenmakers E, Verrijdt G, Peeters B, Verhoeven G, Rombauts W, Claessens F. Differences in DNA binding characteristics of the androgen and glucocorticoid receptors can determine hormone-specific responses. *J Biol Chem.* 2000;275(16):12290-7.

Opportunities for Training and Professional Development

Dr. Shihan He, a postdoctoral fellow, has been responsible for the majority of studies reported here, under my mentorship. He wrote the draft of this progress report, gaining writing experience and learning from my editing. Shortly after starting in the lab, he increased his knowledge of steroid receptor action by attending and participating in the Great Lakes Nuclear Receptor Conference.

Dissemination of Results

Nothing to report

Next Reporting Period

As mentioned above, the selective effects of dox and Cpd05 on androgen-driven gene expression will be examined *in vivo*, both in cells and in mouse xenografts. A broader view of differentially affected genes will be obtained by RNA-seq, and response elements underlying differential regulation revealed by chromatin immunoprecipitation (ChIP) for known genes and genome-wide by ChIP-seq. Results will strengthen our overall hypothesis that genes with similar function (i.e., differentiation or proliferation) have promoters with similar response elements.

4. IMPACT

There is nothing significant to report during this period.

5. CHANGES/PROBLEMS

There have been no significant changes or problems to report during this period.

6. PRODUCTS

There is nothing significant to report during this period.

7. PARTICIPANTS

When this project proposal was submitted, Dr. Pia Bagamasbad was named for the slot for a postdoctoral fellow. She had been in the lab already 2 years and had performed some pilot screening and preliminary characterization of dox activity. Unfortunately we were unable to extend Dr. Bagamasbad's visa and she returned to the Philippines to take a position as an assistant professor, leaving June 13, 2014, just prior to initiation of funding for this project. We were fortunate to recruit another postdoctoral fellow, Dr. Shihan He, who had experience with high-throughput screening and cell-based validation assays. He was not expert in studies of nuclear receptors or prostate cancer and so is receiving training and development in these fields. Thus the individuals on this project are as follows:

Name: Diane M. Robins, Ph.D.
Project Role: P.I.
Research ID (ORCID): 0000-0001-6727-6309
Person Mo.: 1
Contribution: Dr. Robins conceives and oversees the experiments and reports the results
Funding: 10% from this award, 25% from an NCI-RO1, 2.5% from the SPORE

Name: Michele Brogley
Project Role: Res. Assistant
Research ID: n/a
Person Mo.: 1
Contribution: Ms. Brogley assists with cell culture, molecular assays and lab managing
Funding: Ms. Brogley is funded by the RO1 grant

Name: Shihan He, Ph.D.
Project Role: Postdoctoral Fellow
Research ID (ORCID): 0000-0001-5806-8583
Person Mo.: 12
Contribution: Dr. He has performed compound validation and assays to define mechanisms
Funding: Dr. He is funded by this DOD grant

There is nothing to report regarding changes to key personnel funding or to any partner organizations.

8. SPECIAL REPORTING REQUIREMENTS – N/A

9. APPENDICES – N/A