Quantitative Analysis of Androgen Receptor Transcriptional Complexes Using Directed Mass Spectrometry

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Introduction

- Aberrant activity of the androgen receptor (AR) drives the pathogenesis of early- and late-stage human prostate cancer.
- Recent studies have shown that ~50% of prostate cancer harbors gene fusions that place the ETS-family transcription factors under the control of androgen-regulated genes (*ARGs*) (*e.g. TMPRSS2-ERG*)[1].
- The TMPRSS2-ERG fusion proteins promote tumorigenesis by increasing the invasive potential and disrupting normal AR activity to promote differentiation in human prostate epithelial cells[2,3].
- The **goal** of this study is to use quantitative mass spectrometry to identify coregulators of AR-mediated transcription that regulate the expression of ARGs linked to the pathophysiology of human prostate cancers.
- We have developed a workflow to identify these coregulators and this workflow will be used with stable-isotope-dilution multiple reaction-monitoring mass spectrometry (SID-MRM-MS)[4] to study the dynamic behavior of AR transcriptional complexes that are recruited to *ARGs*.

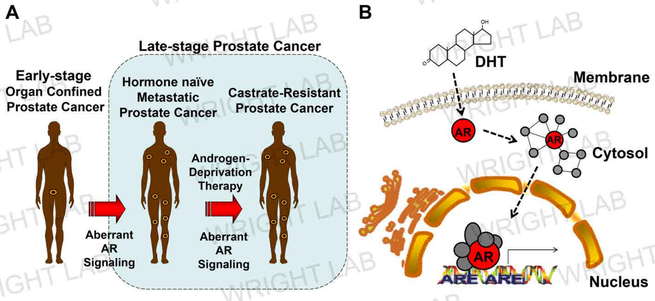

Figure 1. Illustrations of the effects of AR activity. A) Involvement of aberrant AR signaling in early- and late-stage prostate cancers. B) Androgen-mediated AR activation and trafficking in response to androgen exposure.

Methods

- Nuclear protein extracts were prepared from LNCaP prostate cancer cells following androgen-depletion (96 hr in 10% charcoal-stripped FBS + 1 hr with vehicle) or androgen-stimulation (96 hr in 10% charcoal-stripped FBS + 1 hr with R1881).
- The nuclear protein extracts were subjected to affinity-purification with a biotinylated DNA template containing the proximal promoter of the androgen-regulated rat *probasin* gene.
- The *probasin* DNA template had been re-engineered to enable the release of DNA-bound protein complexes by BamHI cleavage and subsequent separation of the complexes by sucrose density-gradient centrifugation.
- The sucrose-separated DNA-protein complexes were analyzed by label-free, directed mass spectrometry (dMS) using Agilent MassHunter Qual on an Agilent 6520 Accurate-Mass Quadropole (QTOF, Agilent, Santa Clara, CA) equipped with a HPLC-Chip-Cube.
- RAW MS files were searched against the SwissProt human database, using Spectrum Mill, for non-redundant protein identifications.

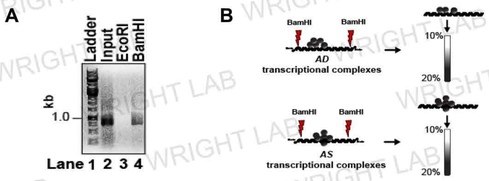

Figure 2. Affinity-purification of AR transcriptional complexes. A) Recovery of *probasin* promoter DNA using restriction enzyme, BamHI. B) Biochemical workflow for DNA-based affinity-purification of androgen-sensitive AR protein complexes.

Results

I.

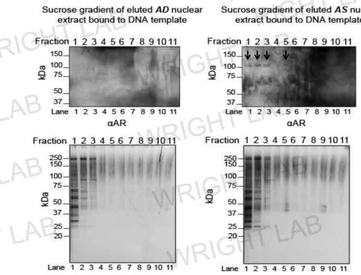

Figure 3. Sucrose density-gradient analysis of affinity-purified androgen-sensitive transcriptional protein complexes. Western blot (anti-AR antibody) and silver stain analysis of samples affinity-purified using the *probasin* promoter. Affinity-purified proteins from androgen-depleted (*AD*) and –stimulated (*AS*) nuclear extracts were subjected to sucrose density-gradient centrifugation and 11 fractions were collected.

II.

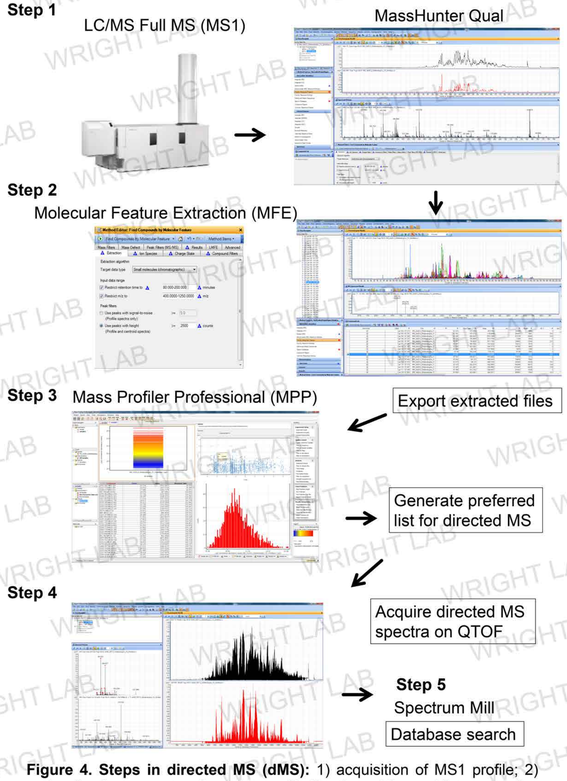

Figure 4. Steps in directed MS (dMS): 1) acquisition of MS1 profile; 2) molecular feature extraction of ions; 3) filtration of ions of interest using Mass Profiler Professional and generation of preferred list for dMS; 4) acquisition of dMS spectra; 5) search against database.

III.

Table 1. Androgen-sensitive AR coregulators

Androgen-sensitive coregulators	Function
AD **NCOA2**	Histone Acetyltransferase[5,6]
AD **NCOR1**	Corepressor[7]
AS **NCOR2**	Corepressor[7]
AS **PRMT1**	Histone Methyltransferase[8]
AS **PRMT5**	Histone Methyltransferase[9]
AD **MED1**	Mediator[10]
AD **ACTN4**	Actin-binding protein coregulator[11]
AD **DYRK1A**	Dual-specificity kinase[12]
AD **RNF6**	E3 ubiquitin ligase[13]

Note: Preliminary analysis of androgen-sensitive AR coregulators identified in sucrose gradient **fraction 1** of *AD* and *AS* samples using label-free, quantitative mass spectrometry.

IV.

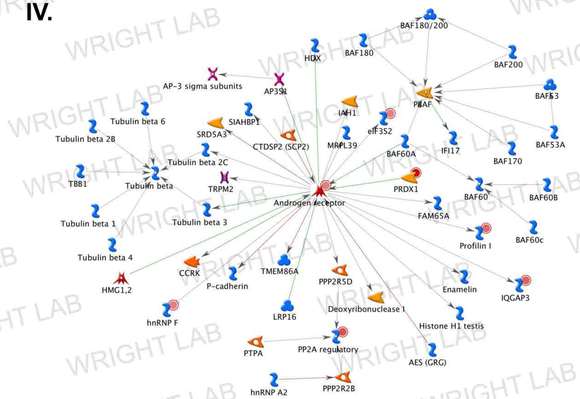

Figure 5. Androgen-sensitive AR protein-protein interaction network identified in sucrose gradient fraction 2 of the *AS* sample. The network comprises proteins involved in the organization of cellular macromolecular complexes, assembly and disassembly of chromatin, disassembly of nucleosomes, and disassembly of protein-DNA complexes.

V.

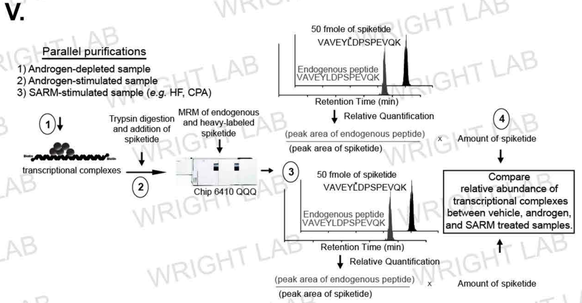

Figure 6. Workflow for comparing the relative abundance of transcriptional protein complexes between vehicle-, androgen-, and SARM-treated samples: 1) affinity-purification; 2) addition of spiketides; 3) multiple reaction monitoring (MRM) of endogenous and heavy-labeled spiketides; 4) comparison of relative abundance of transcriptional complexes between samples.

Future Directions

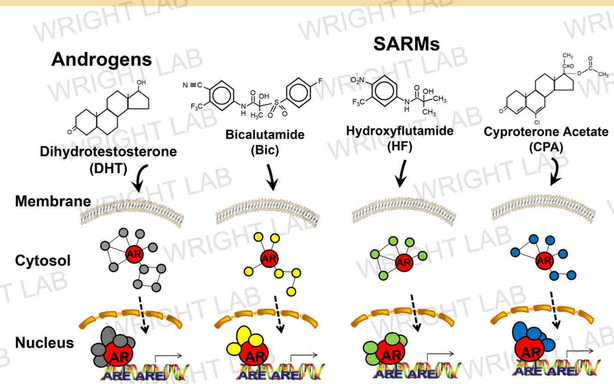

Figure 7. Molecular model of how androgen and antagonistic SARMs may influence AR protein-protein interactions. Our comparative analysis will provide mechanistic insight into the actions of SARMs in prostate cancer cells.

Summary

- We have developed a biochemical strategy to define the androgen-sensitive cofactors of AR-mediated transcription that contribute biochemically to the development of human prostate cancers.
- We have identified both AR-interacting proteins that had previously been implicated in modulating AR transcriptional activity, and AR-interacting proteins with novel roles in this process.
- We will utilize this workflow in combination with SID-MRM-MS methods to assess the dynamic behavior of AR-mediated transcription following exposure to androgens and antagonistic selective androgen receptor modulators (SARMs); we expect to thereby elucidate the molecular mechanism whereby antagonistic SARMs disrupt aberrant AR activity in prostate cancer cells.
- We expect a quantitative model of androgen-mediated transcription to provide a molecular framework for studying the biological actions of SARMs in the therapies for patients afflicted with prostate cancer.

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