Using Label-Free Mass Spectrometry Workflows to Quantitatively Model Signal Transduction Pathways in Cellular Systems and Clinical Samples

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Introduction

Aberrant activity of the androgen receptor (AR) drives the pathogenesis of early- and late-stage human prostate cancers. Approximately 50% of early-stage, localized prostate cancers harbor gene fusions under the control of AR that promote tumorigenesis by increasing the invasive potential of tumor cells and by disrupting normal AR activity to promote terminal differentiation in prostate epithelial cells[1,2,3]. The goal of our research program is to use quantitative proteomics to delineate the dynamic behavior of androgen-signaling networks at the molecular level to understand how aberrant AR signaling drives the development and progression of early- and late-stage prostate cancers. Here we summarize our quantitative proteomic workflows for dissecting AR signaling in model cellular systems of prostate cancer, for identifying prognostic biomarkers in early-stage prostate cancers, and for delineating the mechanisms of drug-resistance in late-stage prostate cancers[4].

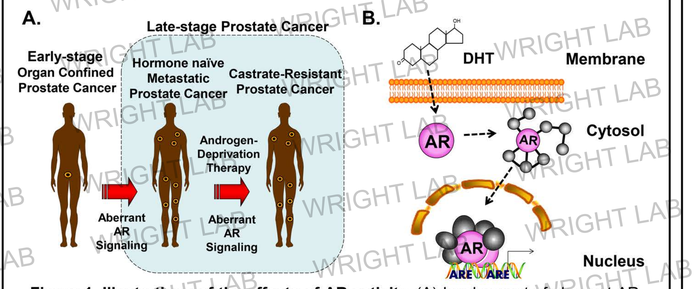


Figure 1. Illustrations of the effects of AR activity. (A) Involvement of aberrant AR signaling in early- and late-stage prostate cancers. (B) Androgen-mediated AR activation and trafficking in response to androgen exposure.

Methods

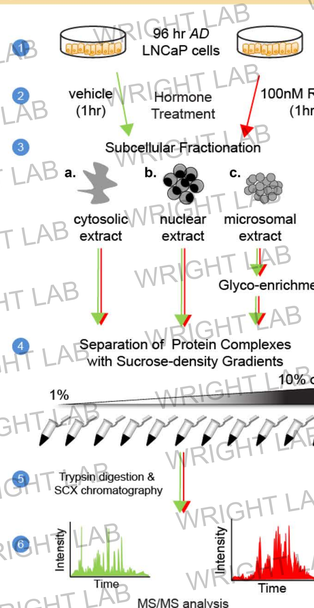


Figure 2. Biochemical workflow for analyzing androgen-sensitive protein complexes across cellular compartments.

1 - 3 : Cytosolic , membrane, and nuclear proteins were prepared from LNCaP prostate cancer cells grown in androgen-depleted media for 96 hr and acutely treated with or without androgen for 1 hr.

3c : Membrane-associated glycoproteins were enriched from the microsomal extracts through lectin affinity-purification using wheat-germ agglutinin (WGA) and concanavalin A (ConA) agarose beads.

4 : The isolated extracts were then subjected to sucrose density-gradient fractionation to separate protein complexes based on their molecular weight.

5 & 6 : The sucrose-separated protein complexes were then analyzed by label-free, directed mass spectrometry (dMS) on an Agilent 6520 Accurate-Mass Quadropole (QTOF, Agilent, Santa Clara, CA) equipped with a HPLC-Chip-Cube.

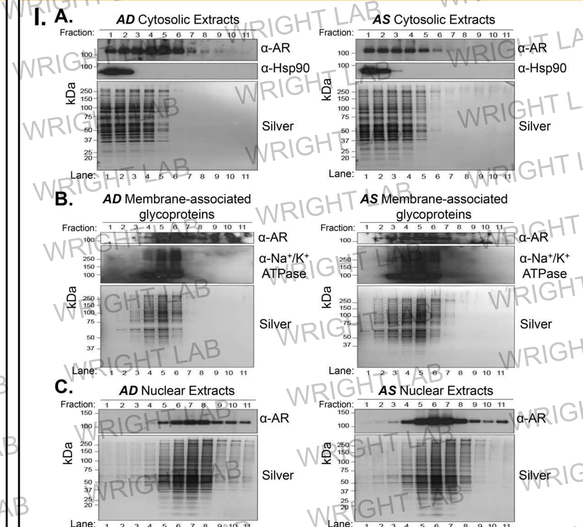


Figure 3. Sucrose density-gradient analysis of androgen-sensitive protein complexes from (A) cytosolic, (B) membrane, and (C) nuclear compartments. Western blot and silver stain analysis of androgen-depleted (*AD*) and –stimulated (*AS*) protein extracts were subjected to sucrose density-gradient centrifugation and 11 fractions were collected.

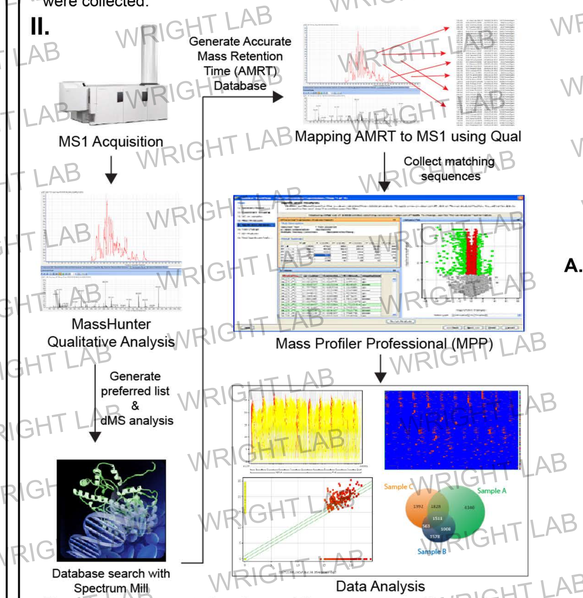


Figure 4. Computational workflow to quantify androgen-sensitive protein complexes across cytosolic, membrane, and nuclear compartments.

Results

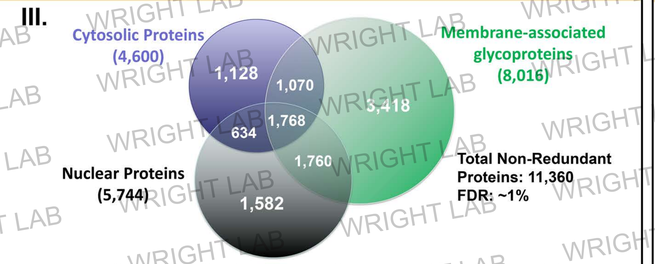
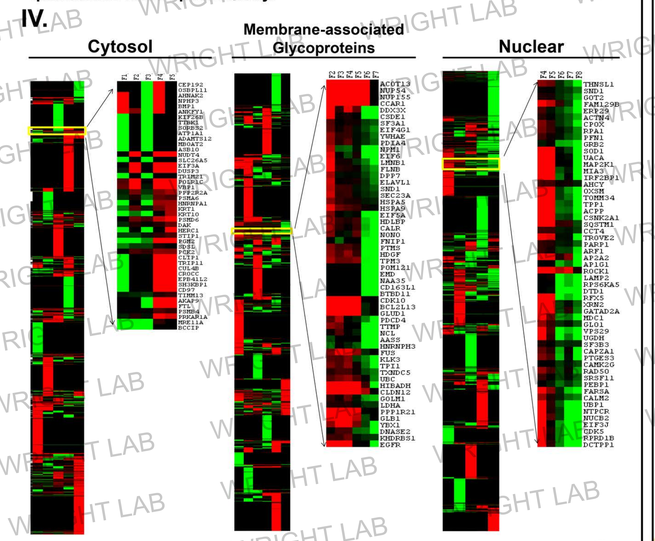


Figure 5. Summary of cytosolic, membrane-associated glycoproteins, and nuclear proteins identified in LNCaP prostate cancer cells using label-free, quantitative mass spectrometry.



Expected Outcomes

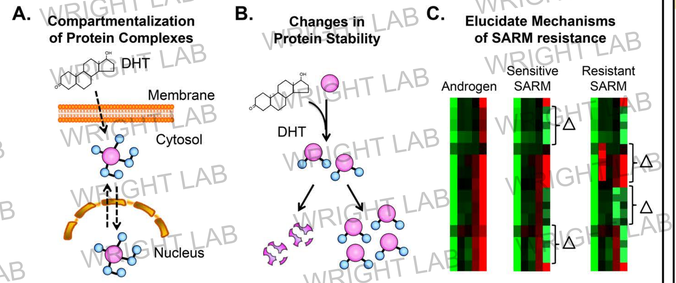


Figure 6. Cluster analysis of androgen-sensitive protein complexes across cytosolic, membrane-associated glycoproteins and nuclear compartments (top panel). Clustered proteins are anticipated to have one or more of the following illustrated outcomes (bottom panel): (A) Changes in the subcellular localization of protein complexes. (B) Changes in protein stability (C) Delineate protein signatures of SARM resistance in prostate cancer. Δ = changes in protein expression.

Future Directions

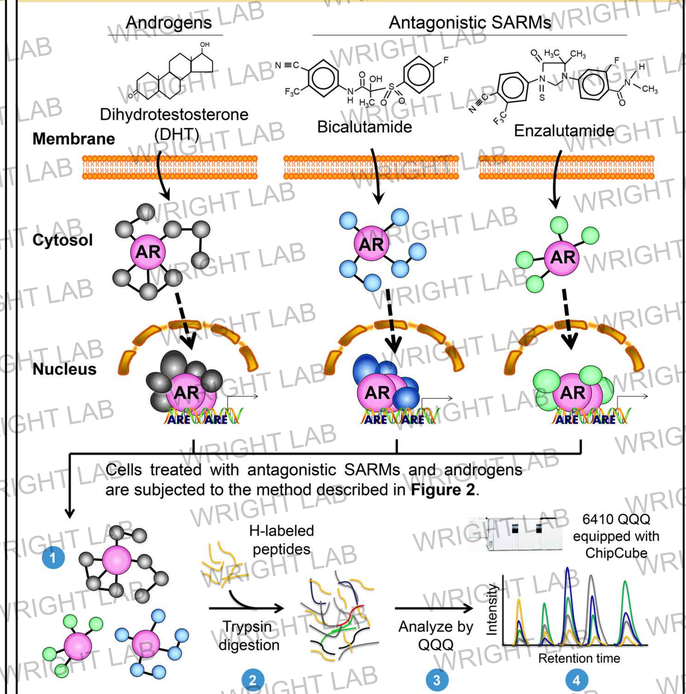


Figure 7. Workflow for comparing the relative abundance of transcriptional protein complexes between vehicle-, androgen-, and SARM-treated samples: 1) isolation of cytosolic, membrane-associated glycoproteins, and nuclear protein complexes through sucrose-density gradient analysis; 2) addition of spiketides; 3) multiple reaction monitoring (MRM) of endogenous and heavy-labeled spiketides; 4) comparison of relative abundance of transcriptional complexes between samples. Our comparative proteomic analysis will provide mechanistic insight into the SARM actions in prostate cancer cells.

Summary

- We have developed a biochemical strategy to define the compartment specific, androgen-sensitive protein complexes that activate molecular and cellular processes in prostate cancer cells in response to an acute exposure to androgens.
- We have found the localization and abundance of AR coregulators change in response to androgens, and suggest AR coregulators are tightly controlled in a spatial and temporal dependent manner.
- We will utilize this workflow in combination with SID-MRM-MS methods to assess the dynamic behavior of androgen-sensitive protein complexes 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.
- Lastly, we expect a quantitative model of androgen-induced signaling cascade to provide a molecular framework for studying the biological actions of SARMs in the therapies for patients afflicted with prostate cancer.

References:

1. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, Varambally S, Cao X, Tchinda J, Kuefer R, Lee C, Montie JE, Shah RB, Pienta KJ, Rubin MA, Chinnaiyan AM (2005). Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science 310*, 644-648
2. Tomlins SA, Laxman B, Varambally S, Cao X, Yu J, Helgeson BE, Cao Q, Prensner JR, Rubin MA, Shah, RB, Mehra R., Chinnaiyan AM (2008). Role of the TMPRSS2-ERG gene fusion in prostate cancer. Neoplasia *10*, 177-188.
3. Helgeson BE, Tomlins SA, Shah N, Laxman B, Cao Q, Prensner JR, Cao X, Singla N, Montie JE, Varambally S, Mehra R, Chinnaiyan AM (2008). Characterization of TMPRSS2:ETV5 and SLC45A3:ETV5 gene fusions in prostate cancer. Cancer Res *68*, 73-80.
4. Lange V, Picotti P, Domon B, Aebersold R (2008). Selected reaction monitoring for quantitative proteomics: a tutorial. Mol Syst Biol *4*, 222.

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