Identification of the Protein Expression of Androgen Receptor Variants Using Targeted Proteomics in Clinical Castration Resistant Prostate Cancer Models

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Background

Castration Resistant Prostate Cancer (CRPC) is a treatment resistant form of prostate cancer (PCa). Currently, there is not a way to identify which patients will develop this resistance before full blown CRPC develops. Therefore, current PCa treatment approaches are similar. However, this results in tumor regression in some cases and progression in others. Targeting the androgen receptor (AR) is the main focus of current therapies even in CRPC. Emergence of AR splice variants (AR-Vs) after initial treatment is thought to be one of the primary resistatnce. AR-Vs lack the ligand binding domain rendering Androgen Deprivation Therapy (ADT) ineffective in tumors expressing these variants. Recent work has identified the DNA and RNA species of ARVs in CRPC but investigation into whether the protein is translated are unknown. One exception is the approval of an antibody test that detects a specific AR-v, AR-v7, from the blood of PC patients and predicts ADT response. However, there are instances where a patient may not express ARv7 and are still resistant to ADT. This suggests that other AR-vs, not currently detected at the protein level, are potentially important predictors for ADT response. The primary goal of this effort is to expand the protein identification of known and unknown AR-Vs that may assist in the response to ADT using a targeted liquid chromatography tandem mass spectrometry (LC-MS/MS) approach. LC-MS/MS can provide accurate, precise, sensitive, and reproducible detection of a pre-determined set of peptides in specimens without the need for antibody enrichment.

Objective: The goal of this work is to generate a robust set of unique peptides that behave as surrogates for AR-V proteins, analyze them via targeted LC-MS/MS approach and screen clinical samples from prostate cancer patients.

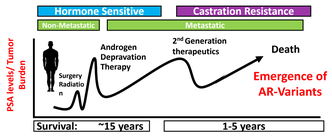


Figure 1. Progression of Drug Resistance in CRPC

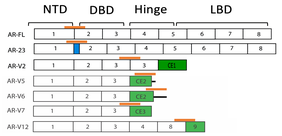
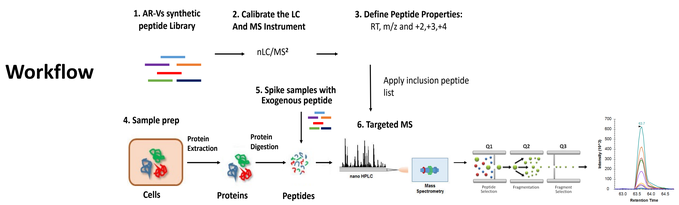


Figure 2. Schematic overview of AR-Variants are Associated with Drug Resistant Mechanism. N-Terminal Domain (NTD), DNA Binding Domain (DBD), Ligand Binding Domain (LBD).

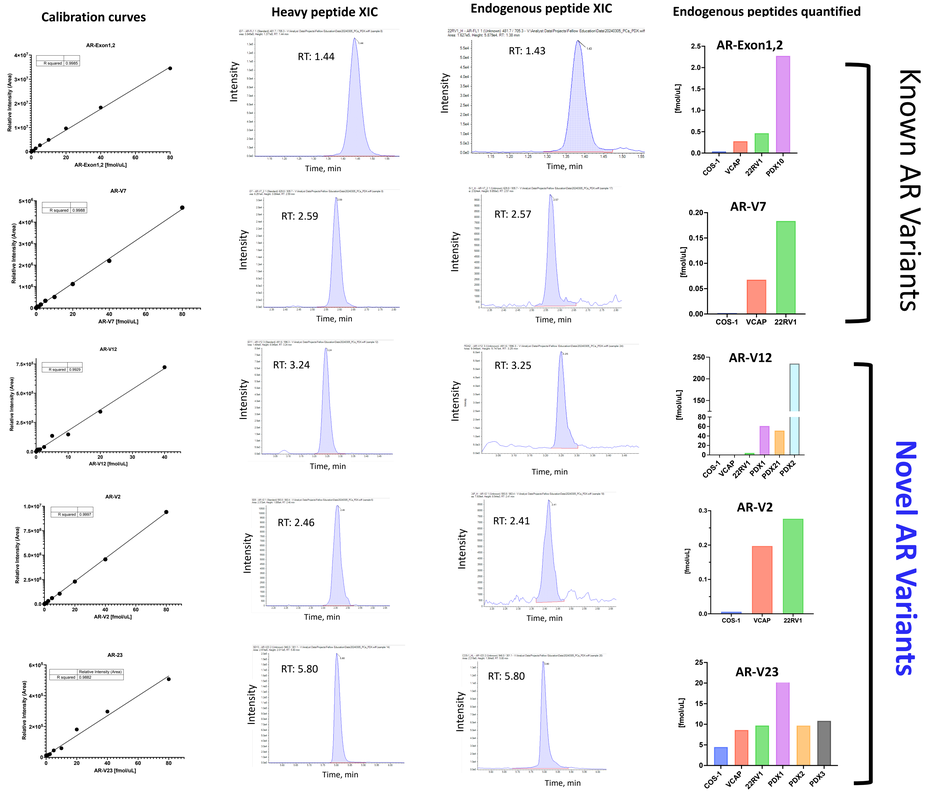
Method

We developed the AR-v assay by first identifying AR-vs from unique splice regions via RNA-guided sequences translated to amino acid sequences. The AR-variants amino acid sequences were then in-silico trypsin digested using Expasy software to generate tryptic peptides followed by blast analysis of the AR-v peptides where no overlap with other human proteins was observed. The unique variant was then further analyzed by with Skyline software to generate extracted ion chromatograms visuals. These AR-v peptide sequences were commercially synthesized along with heavy isotopically labeled analogs to be used as internal standards in the LC-MS/MS method. The LC-MS/MS system consisted of a XR HPLC system (Shimadzu) with a QTRAP 6500 triple quadrupole mass spectrometer (SCIEX) using electrospray ionization and multiple reaction monitoring (MRM) for analysis. Experiments were conducted under high-flow conditions with a flow rate of 0.700 mL/min and a 7-minute run time employing gradient chromatography. Mobile phase solutions consisted of 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B). A Kinetex (Phenomenex) 2.6 um C-18 100 A 50 x 3 mm column was used for all chromatographic runs. The method examined 9 peptides and included 2 to 3 MRM transitions per peptide. Calibration curves were constructed using unlabeled peptides over 0.10 pmol/uL to 1 pmol/uL. Cell lines examined consisted of a panel of prostate cancer cell lines (n= 6) were, CRPC patient derived xenografts tumors (n=48) and a negative control cell line (no androgen receptor expression), Cos-1, which served dual function and was used as matrix for peptide MRM optimization experiments. Cell line specimens were processed using lysis buffer containing 7 M of urea, 2 M thiourea, 0.4 M Tris pH 8.0, 20% acetonitrile (ACN), 10 mM TCEP and 25 mM chloroacetamide. Protease inhibitor HALT added to the lysis buffer at 1x concentration immediately before addition to the cell line specimens. Viscous specimens, likely due to chromatin release, were sonicated using a probe sonicator set at 30% amplitude, for 5 seconds while samples were on ice. The specimens then underwent proteolysis using LysC pH 8.2 incubated at 37ºC for 4 hours and then trypsin at pH 8.2 incubated at 37ºC in a warm air incubator overnight (~16 hrs). After incubation, the samples were acidified with 10% trifluoroacetic acid. Specimens were desalted using reverse phase HLB columns and the elutates were dried down using a speed vacuum. Samples were re-suspended with 0.1% formic acid. Internal standard solution was spiked-in to each calibrator and cell line specimen and then added to a 2 mL autosampler vials with 300 µL glass inserts. Linearity and sensitivity for each peptide included in the method was examined using calibration curves. Data analysis was conducted using Multiquant (SCIEX) software (Figure 3.)

Results



AR variants Expression in Prostate Cancer Cell lines and PDX samples



Representative chromatograms are shown above with corresponding retention time (RT), extracted ion chromatogram (XIC), cell lines and patient derived xenograft (PDX) samples.

Conclusions

- Designed and established a peptide library that identifies 5 AR variants.
- We are monitoring a total of 16 transitions with 2-3 transitions per AR-Vs.
- Generated an inclusion list that measures 7 AR-Vs.
- Establish a platform to measure AR variants landscape.
- Identified protein expression of **3 novel AR-Vs** (2, 12 and 23) in cell lines and PDX tumor samples from Castration Resistant Prostate Cancer patients.
- We have developed calibration curves on all the AR-Vs.

Future Directions

- Optimize the AR-V5 and AR-V6 transitions and calibration range.
- Conduct method validation studies by performing imprecision, stability, linearity, and recovery studies.
- Continue testing different prostate cancer cell lines and other specimen types such as urine, extracellular vesicles, etc.

References

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