Discovery of candidate tumour markers for prostate cancer via proteomic analysis of cell culture conditioned media

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ABSTRACT:

Introduction:

- Prostate cancer (CaP) is the most common malignancy and the second leading cause of cancer-related death in men in the United States.
- Early detection and treatment of clinically relevant prostate tumours (CaP) significantly improves the survival of prostate cancer patients.
- Prostate specific antigen (PSA) is currently used for as a screening tool for the early detection of CaP, albeit it suffers from low specificity and is unable to distinguish aggressive tumours.
- There is, therefore, a need for more specific tumour markers for early detection of aggressive prostate cancer that can provide a prognosis of the patient after an initial diagnosis is made.

Purpose:

In this study, our objective was to perform proteomic analysis of conditioned media from the prostate cancer cell line $PC3(AR)_6$. The secreted and membrane proteins will then be investigated as candidates for their potential as biomarkers for prostate cancer.

Methods:

- $PC3(AR)_6$ was cultured in serum-free media for 14 days using a roller bottle culture system.
- The conditioned media was fractionated by strong anion-exchange chromatography.
- The collected fractions were trypsinized and analysed by reversed phase C-18 chromatography tandem mass spectrometry.
- The mass spectra generated were searched using the MASCOT database and identified proteins were organized based on their genome ontology classification of cellular location.

Results and Conclusion:

- A total of 262 proteins were classified according to cellular location and the sample was found to contain a significant proportion of secreted and membrane proteins.
- Protein X was identified and was measured by ELISA in serum samples from CaP patients and healthy males and shown to be elevated in a cohort of CaP patients

BACKGROUND:

- The enthusiasm over early detection of CaP centers on the fact that if diagnosed early, before it has breached the capsule of the prostate, the organ may be treated with radiation or chemotherapy or removed by radical prostatectomy.[1]
- Recently there has been renewed interest in the search for cancer biomarkers for early detection, and this has come about mainly as a result of the introduction of mass spectrometry for the analysis of complex mixtures of proteins.
- Currently, many people have used a variety of proteomic approaches to look at serum, tumor tissues, cell culture supernatants and other bodily fluids, in the hopes of finding discriminating proteins that could be useful as biomarkers.[2, 3, 4, 5]
- However, due to the complexity of biological fluids, the elucidation of low abundant proteins through proteomics is a considerable challenge.
- Our aim is to use the prostate cancer cell line $PC3(AR)_6$ grown in serum free media as a model system to identify secreted proteins. These may be strong candidates as biomarkers for CaP.

OBJECTIVES:

- Culture $PC3(AR)_6$ prostate cancer cell line in a large volume over an extended period of time
- Determine optimal growth time to maximize amount of secreted protein
- Analyze fractionated conditioned media by ESI-MS/MS to identify proteins present
- Organize identified proteins by cellular localization and determine candidates
- Validate Protein X and others as serological markers for prognosis and diagnosis of CaP

METHODOLOGY:

Cell Culture

- $PC3(AR)_6$ cell line
  - Grown in 2 X 175cm² in RPMI 1640 + 8% FCS
  - Transferred to 850cm² roller bottle
  - Media changed to CDCHO serum-free (400mL) after 48 hours
  - Cultured for 14 days
  - Collected conditioned media and dialyzed at pH 8.9 – 20mM Diethanolamine

Strong Anion Exchange Chromatography

- Fast Performance Liquid Chromatography (FPLC)
- Dialyzed media was directly loaded onto a SAX column
- Elution – linear two stage gradient
  - 0 M to 0.6 M NaCl – 40 mins
  - 0.6M to 1M Nacl – 20 mins

Lyophilization

- Fractions were lyophilized to dryness O/N

Trypsin Digestion

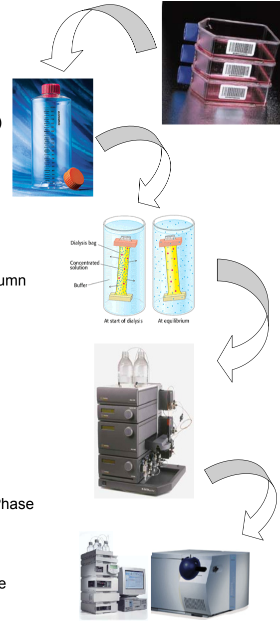
- Each fraction was trypsin digested O/N

C-18 HPLC – ESI-MS/MS

- Desalted and purified via C-18 ZipTiP
- Eluted from ZipTiP and fractionated via Reversed Phase chromatography C-18
- Coupled online to an ESI-MS/MS

Mascot Search

- Each batch was searched independently against the Mascot search database



RESULTS:

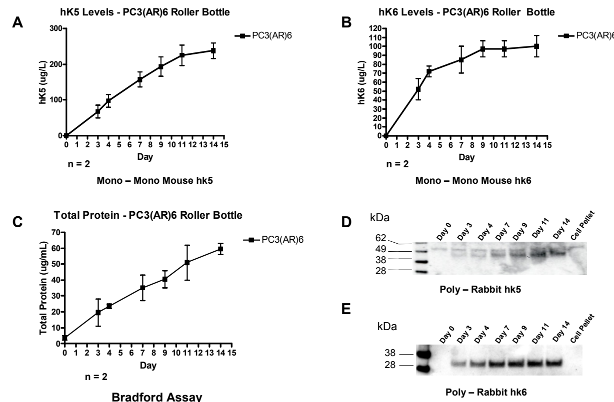


Figure 1: hK5, hK6 and Total Protein measured over time in $PC3(AR)_6$ conditioned media. (**A**) ELISA results measuring hK5 and (**B**) hK6 over time in $PC3(AR)_6$ conditioned media. (**C**) Bradford assay total protein results of $PC3(AR)_6$ conditioned media over time, and (**D**) Western blots of hK5 and (**E**) hK6 over time of $PC3(AR)_6$ conditioned media, and cell pellet – Note – No hK5 or hK6 is present in the cell pellet.

RESULTS (cont.)

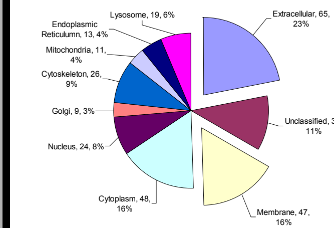


Figure 2: Classification of proteins by cellular location from $PC3(AR)_6$ conditioned media Batch #1 and #2. Shown is the distribution of the different cellular localization classifiers of the proteins identified. In total 262 proteins were identified. 39% are classified as extracellular or membrane and 50% are classified as intracellular.

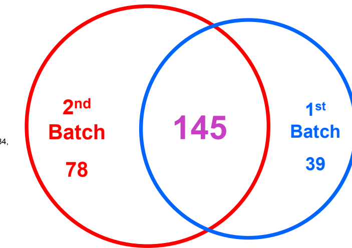


Figure 3: Venn diagram of overlap of proteins identified by MS between Batch #1 and #2. 145 proteins (55%) are common between to the two batches. This can be attributed partially to differences in sample preparation of the two batches and mainly to selective ionization during the MS step.

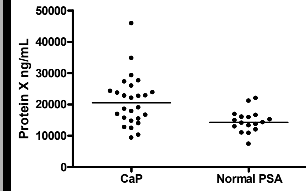


Figure 4: Protein X serum levels in CaP vs. healthy males measured by ELISA (median value shown)

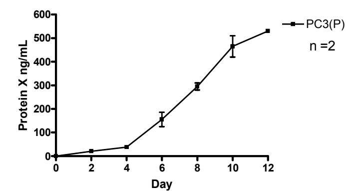


Figure 5: Protein X levels in PC3(P) conditioned media measured by ELISA

SUMMARY AND CONCLUSIONS:

- The $PC3(AR)_6$ cell line was cultured in large volume over an extended period
- Total protein and secreted protein markers hK5 and hK6 were monitored over time in the culture media and were seen to increase steadily
- We validated our sample preparation and MS method by identifying our positive control proteins hK5, hK6 in the conditioned media via ESI-MS/MS
- A total of 262 proteins were identified to be present in the conditioned media by ESI-MS/MS
- The identified proteins were organized based on their cellular localization
- 39% are putative secreted or membrane proteins, from these candidates will be chosen
- Protein X is currently being examined as a serological biomarker for prognosis and diagnosis of prostatic carcinoma

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