Proteomic analysis of culture supernatants from the prostate cancer cell line PC3(AR)_6

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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. The etiology of CaP is currently unclear, making primary prevention unfeasible. Therapies for advanced stages of CaP are elusive. Early detection and treatment of prostate tumors that are localized to the prostate significantly improves the clinical outcome of CaP patients. Measuring prostate specific antigen (PSA) levels in blood is widely used for early detection of CaP, albeit it suffers from low specificity since it is more indicative of the size of the prostate, which may be due to benign prostate hyperplasia, rather than of the cancer itself. There is, therefore, a need for more specific tumour markers for early detection of prostate cancer.

Purpose: In this study, our objective was to perform proteomic analysis of conditioned media (CM) from the prostate cancer cell line PC3(AR)_6. The secreted and membrane proteins may represent candidate biomarkers for prostate cancer.

Methods: The prostate cancer cell line PC3(AR)_6 was cultured in large volumes (400mL) of chemically defined serum-free media for 14 days in two batches. The conditioned media were then collected and processed separately. They were dialysed at pH 8.9 and the dialysed media were fractionated by anion-exchange chromatography and the collected fractions were lyophilized and trypsin digested. The tryptic peptides were further fractionated by reversed phase chromatography before being analyzed by electrospray ionization tandem mass spectrometry (ESI-MS/MS) and Mascot database searching. The proteins identified from both batches were combined and organized into subgroups based on their gene ontology classification of cellular location.

Results and Conclusion: A total of 360 proteins were classified according to cellular location and the sample was found to contain a significant proportion of secreted or soluble proteins. Systematic proteomic and bioinformatic analysis of the secreted proteome of cell lines may reveal proteins which could be biomarkers of prostatic and other carcinomas.

BACKGROUND:

The enthusiasm over early detection of CaP centers on the fact that 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.

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.

Our aim is to characterize the secreted proteins from the prostate cancer cell line PC3(AR)_6 grown in serum free media. Since 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.

Monitor levels of Kallikreins 5 (hK5) and 6 (hK6) over time by ELISA.

Determine optimal growth time.

Maximize amount of secreted protein.

Fractionate conditioned media via strong anion exchange.

Analyze conditioned media by ESI-MS/MS.

Identify hK5 and hK6 by mass spectrometric analysis of the fractionated conditioned media.

Organize identified proteins by cellular localization.

METHODLOGY:

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)
- Cultured for 14 days
- Collected conditioned media and dialyzed at pH 8.9 – 20mM Diethanolamine

Strong Anion Exchange Chromatography

- Fast Performance Liquid Chromatography (FPLC)
- Dialed media directly loaded onto a SAX column
- Elution – linear gradient – 0 to 1 M NaCl

Lypophilization

- 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 chromatogtaphy C-18
- Coupled online to an ESI-MS/MS

Mascot Search

- Each batch was searched independently against Mascot search database

RESULTS:

Strong Anion Exchange Chromatography

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SILVER STAIN

- Bradford assay
- Silver stain
tected proteins from the prostate cancer cell line PC3(AR)_6 conditioned monitored to determine optimal secretion levels of hK5 and hK6.

References:


SUMMARY AND CONCLUSIONS:

- The PC3(AR)_6 cell was cultured in large volume over an extended period.
- Total protein and secreted protein markers hK5 and hK6 were monitored over time in culture media.
- 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 360 proteins were identified to be present in the conditioned media by ESI-MS/MS.
- The identified proteins were organized based on cellular localization.
- 40% are putative secreted or membrane proteins.
- These candidates are currently examined and serological biomarkers for prostatic carcinoma.

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