In Vitro Proteomic Profile Analysis of Metastatic Prostate Cancer By MALDI-TOF-TOF MS

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Overview

A proteomic profile analysis of metastatic prostate cancer was performed utilising MALDI-TOF-TOF-MS

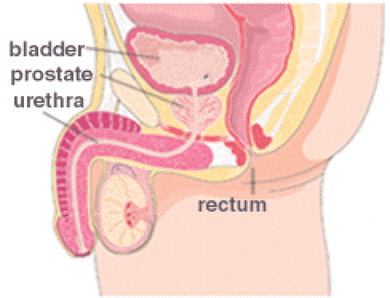

Figure 1: Anatomy of the male reproductive system showing the prostate gland

Introduction

Prostate cancer (PC) is the second leading cause of cancer-related death in American males. Although androgen deprivation attenuates localized PC, relapse of androgen independent disease ultimately dictates PC related morbidity and mortality. It has been suggested that the differential proteomic approach is valuable for mass identification of differentially expressed proteins involved in prostate tumorigenesis.

Methods

Cell Culture

The PC-3 cells originally isolated from vertebral metastases from prostate cancer patients, and the RWBE1cells, an established normal prostate epithelial cells were obtained from the American Type Culture Collection (ATCC). All the PC cell lines will be passaged with Trypsin-EDTA and allowed to grow to confluency over 4-5 days. Both cell lines were maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1 % penicillin-streptomycin and 1 % L-glutamine, and grown in a humidified incubator at 37oC and 5% CO2.

Cytoplasmic Protein Extraction

Total cell lysates were prepared from 70% confluent bone metastasis prostate cancer cell line PC-3 and normal prostate epithelium cell line RWBE1 for comparison. Cytoplasmic extracts were performed according to the manufacturer's recommendation. Briefly, cells were harvested by scraping or by trypsinization, washed and pelleted by centrifugation. All subsequent manipulations were performed on ice in presence of protease inhibitors (PMSF, aprotinin, leupeptin and benzamidine) in the cytoplasmic extraction reagent. (CER I). Packed cell volumes (PCVs) were estimated to determine the appropriate volumes of extraction reagents to be used. For a 20 ul packed cell volume (~40 mg), 200 ul CER I was added and the cell pellet was vortexed for 15 seconds to fully resuspend the cells. The cells were incubated in the presence of 11 ul CER I and were further incubated for 1 minute. The lysed cells were centrifuged for 5 minutes at maximum speed in a microfuge to pellet the intact nuclei. The supernatant was carefully removed (cytoplasmic fraction) and stored at -80C.

Western blot analyses of PC3 and RWBE1 cells.

Equivalent amounts of total cytoplasmic protein (20 g) were loaded in each lane of a 1-DE 4-20% gradient Tris-Glycine denaturing gel (Promega). The advantage of 1-DE separation system is that it is usually carried out with sodium dodyecyl sulfate (SDS), in which most proteins are soluble, and that both acidic and basic proteins can be separated and easily visualized. The resolved proteins were then visualized using Sypro Ruby stain (Bio-Rad). After electrophoresis, gels were washed in 10% methanol/7% acetic acid for approximately 1 hr, then placed in Spyro Ruby for at least 3 hrs, and finally destained for at least 1 hr. Bands representing differentially expressed genes were excised under UV for subsequent analysis by MALDI-TOF-TOF MS.

Protein Digestion and Mass Spectrometry Analysis.

Proteins were digested at UTMB using their protocols. Mass spectrometry was performed on an ABI 4700 and a LaserToF TT (Tof-Tof) (SAI Ltd.).

Results

Peptide mass analysis and database comparison have demonstrated differential expression of the following proteins in metastatic prostate cancer cells, PC-3, but not the normal prostate epithelial cells. The detected proteins are as follow:

Probability	***Name***	***%***	***pI***	***kDa***
1.00E+000	Ig mu chain precursor	18	5.8	68.5
1.90E-001	(XM_052186) KIAA0411 gene product	16	5.9	35.2
3.90E-001	collagen alpha 2(XI) chair precursor	6	8.7	151.0
2.90E-001	heat shock 10 kD protein (Chaperonin 10)	55	8.9	10.9
1.90E-001	Chain A, Human cyclophilin A	25	8.1	17.9

Table 1: Detected proteins unique to PC-3 cells

Discussion

Five proteins unique to the cancer cell PC-3 were identified. The presence of these proteins shows that the cell is in a high state of readiness (increased cell signaling activity) and action for protein expression and folding, as evidenced by the presence of chaperonins and immunophilins. The presence of Ig mu is confusing since PC-3 cells are not B cells. This could only be explained if the sequence detected was part of the Ig superfamily which includes growth factors and kinase receptors.

Conclusion

The findings illustrate the potential of proteomics as an effective technique for identifying differentially expressed proteins in prostate cancer cells with aggressive phenotype. These data can be used to establish a proteome database to further study human prostate tumorigenesis. We are currently pursuing experiments to unravel the functional significance of these proteins as to whether they can be used as biomarkers or if they play a role in the pathogenesis of disease progression. In addition, our data should strengthen our basic understanding of metastatic PC and provide a new frontier for identifying relevant preventive and/or therapeutic targets for disease progression.