Abstract: S402

Proteomic Profiling of Prostate Cancer and Benign Prostatic Hyperplasia from Urine

A.Haj-Ahmad[1], M. Abdalla[2] and Y. Haj-Ahmad[1,2]
[1]Department of Biological Sciences, Brock University, 500 Glenridge Avenue, St. Catharines, ON, L2S 3A1, Canada
[2]Norgen Biotek Corp. 3430 Schmon Parkway, Thorold, Ontario, L2V 4Y6, Canada

Abstract

Prostate cancer is the most frequently occurring cancer and is the second highest cause of cancer mortality in males. Serum prostate specific antigen (PSA) is currently used as an indicator for the diagnosis and management of prostate cancer. Patients with a serum PSA between 2.5 ng/mL and 10 ng/mL will often undergo prostate biopsies to confirm prostate cancer. However, <30% of these men will have positive biopsies, meaning that the majority of men underwent these invasive investigations with little benefit. The development of new diagnostic tests is therefore required to increase diagnostic accuracy. The objective of this study was therefore to compare the urinary proteome of patients with Prostate Cancer (PCa) as well as Benign Prostatic Hyperplasia (BPH) to the urinary proteome of normal healthy individuals in the hopes of discovering a novel and reliable non-invasive urinary biomarker for the early detection of PCa. Urinary proteins were isolated from urine samples collected from 8 PCa patients, 12 BPH patients or 10 healthy individuals. Total urinary proteins showed significant differences when resolved by SDS-PAGE. These proteins were significantly higher in both PCa and BPH patients, in comparison to those isolated from the healthy individuals. The bottom-up proteomic approach was used to investigate the differential protein expression among the three groups. The preliminary analyses from the LC/MS/MS data identified 85 different proteins that are differentially expressed among the three groups. These proteins might be of great value for the early and accurate detection of prostate cancer from non-invasively collected urine samples.

Introduction

A major goal in the field of clinical proteomics is to identify disease biomarkers in biological fluids that can be measured relatively inexpensively for the early diagnosis of disease. An important challenge in this process is to develop a rational means of reducing the complexity of the proteome of the sample to enhance the detectability of low-abundance proteins that may have a pathophysiological significance (1).

Urine offers a great chance for the development of novel, non-invasive assays for the diagnosis, monitoring and early detection of disease and has the advantage of being noninfectious for HIV and other pathogens (2).

As a specific filtrate of the blood, the protein components of urine are qualitatively similar to those of blood. A catalog of these proteins will not only improve our knowledge of kidney physiology (3), but may also allow the identification of novel proteins associated with pathological states (4).

Prostate cancer (PCa) is the most common cancer among Canadian men (5). In 2008, an estimated 24,700 Canadians were diagnosed with PCa with 4,300 deaths.

A biopsy is currently the only way to confirm the presence of CaP (6). Since 1987, prostate-specific antigen (PSA) has been the most important tumour marker in urology for PCa diagnosis. The disadvantage of the PSA blood test is the false positive results (of 10 men with elevated PSA levels, only 3 will actually have prostate cancer as determined by the first biopsy) and the false negative results (of 5 men with CaP, 1 or 2 will have a normal PSA).

The objective of this study is therefore to elucidate the urinary proteome of patients with PCa as well as BPH in comparison to the urinary proteome of normal healthy individuals. This may lead to the discovery of a novel and reliable non-invasive urinary biomarker for the early detection of PCa, where therapeutic intervention can be more efficient.

Methods

-Urine samples were collected from the Alexandria University General Hospital (Egypt) from 8 PCa patients, 12 BPH patients and 9 healthy individuals (control group)

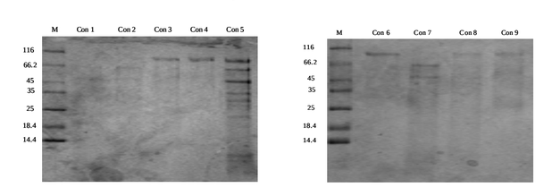
-Total proteins were isolated from 1 mL of urine using the ProteoSpin Urine Protein Concentration Kit (Norgen Biotek Corp.)

-Protein samples were quantified using the Bio-Rad Protein Assay and 20 µL of the 100 µL elutions were run on 15% SDS-PAGE for qualitative analyses

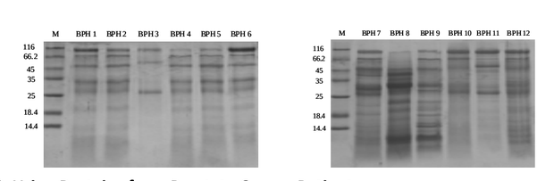
-Mass spectrometry was performed at Sick Kids Hospital (Proteomic Facility, Toronto, ON) using an online LC-MS/MS set up on an Agilent 1100 Capillary LC System (Palo Alto, CA) fitted to an LTQ ion trap mass spectrometer (Thermo Electron, San Jose, CA)

Results

A. Urine Proteins From Healthy Individuals



B. Urine Proteins from Benign Hyperplasia Patients



C. Urine Proteins from Prostate Cancer Patients

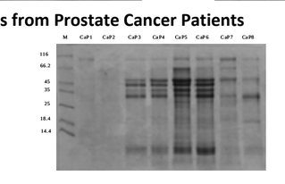
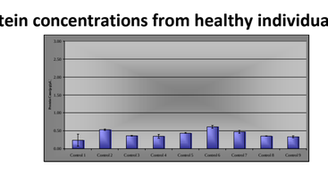
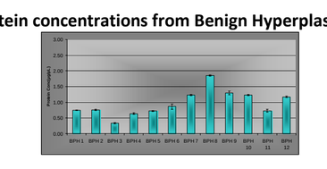


Figure 1. SDS-PAGE of total proteins isolated from 1 mL of urine from healthy individuals (Panel A), Benign Hyperplasia (Panel B) and Prostate Cancer (Panel C). A total of 20 µL of each 100 µL was loaded on a 15% SDS-PAGE gel and run at 200 V for 75 minutes. Lane M contains the Unstained Protein Molecular Weight Marker (Fermentas). Gel photos were taken using an AlphaImager 2200 (AlphaInnotech).

A.Urine protein concentrations from healthy individuals



B.Urine protein concentrations from Benign Hyperplasia patients



C.Urine protein concentrations from Prostate Cancer patients

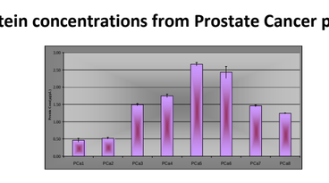


Figure 2. Total protein concentrations from 1 mL urine samples of healthy individuals (Panel A), Benign Hyperplasia patients (Panel B) and Prostate Cancer (Panel C). Total proteins were quantified using the Bradford Assay (BioRad).

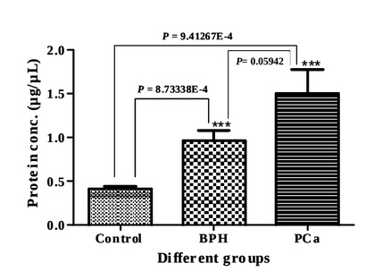


Figure 3. Histogram showing the urinary protein concentrations (mean ± CI) purified from the healthy group (control), Benign Hyperplasia group (BPH) and the Prostate Cancer group (PCa). The means of the urinary protein concentrations from both BPH and PCa groups were significantly different than that from the healthy individuals at $p < 0.05$.

Table 1. Proteins identified by LC-MS/MS and were over-expressed among BPH and Prostate Cancer patients when compared to the control group.

Identified proteins	Control	BPH	PCa
Tumour protein p53 inducible nuclear protein 2	0%	100%	100%
N-ethylmaleimide-sensitive factor attachment protein	0%	99%	100%
Immunoglobulin light chain variable region	0%	100%	88%
Nidogen 1 precursor	0%	89%	88%
CTCL tumour antigen se89-1	0%	89%	88%
Alpha-1-acid glycoprotein 1	0%	89%	88%
Chain A, apo-human serum transferrin (non-glycosylated)	0%	89%	88%
Protein Rei, Bence-Jones	0%	85%	88%

Conclusions

Due to the limitations of the PSA test there is an urgent need for new prognostic biomarkers to enhance the clinical management of prostate cancer. This study identified 8 proteins which were overexpressed among both benign prostate hyperplasia and prostate cancer patients. In addition to the tumour related proteins identified several other proteins may play a direct role in the development of prostate cancer. Vesicular transport proteins (which function in protein synthesis) such as N-ethylmaleimide-sensitive factor attachment protein, have also been demonstrated to be upregulated in colorectal cancer cells and may therefore represent a novel biomarker for several different types of cancer (7). The overexpression of an immunoglobulin is not unexpected as these proteins have recently been found in a variety of cancer cells (8). Preliminary data suggests that the Ig secreted by epithelial cancer cells has some unidentified capacity to promote the growth and survival of tumour cells. As these proteins are overexpressed in BPH patients with negative biopsies they may represent a potential prognostic biomarker for the early detection of prostate cancer when the disease is most amenable to a positive treatment outcome.

References

1. Pisitkun T, Shen R-F, and Knepper MA. (2004). PNAS 101: 13368-13373.
2. Botezatu I, Serdyuk O, Potapova G. *et al*. (2000). Clin Chem. 46: 1078–1084.
3. Thongboonkerd V, Klein JB, Pierce WM, et al. (2003). Am J Physiol Renal Physiol 284: F1155-F1163.
4. Knepper MA. (2002). J Am Soc Nephrol 13:1398-1408.
5. Canadian Cancer Society. (2009). Can. Cancer Statistics. www.cancer.ca/statistics.
6.Schroder, F. H. (2009). Recent Results Cancer Res. 181: 173-82.
7.Tan, H. T., S. Tan, Q. Lin et al. 2008. Mol. Cell. Proteomics. 7: 1174-85.
8.Chen, Z., X. Qui and J. Gu. 2009. Am. J. Pathol. 174: 1139-48

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Brock University
NORGEN BIOTEK CORPORATION