Urinary Protein Profiles in Healthy Individuals, Patients with Proteinuria and Histoplasma Antigenuria

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Overview
- The incidence of pathogenic and opportunistic fungal infections has been increasing over the past decade due to medical advances that prolong lives of the severely ill patients.
- One of the complications of fungal infections can be proteinuria, condition characterized by increased urinary protein excretion.
- The goals of our study were to compare urine protein profiles among healthy individuals, patients with proteinuria who tested positive for histoplasma antigen, and patients with proteinuria who tested negative for histoplasma antigen.
- We identified proteins in common among the evaluated conditions and determined the genes and physiological pathways associated with the excreted proteins.

Introduction
Kidneys remove waste products and toxins from the blood while preventing loss of proteins in the urine. When glomeruli are damaged, proteins appear in urine. In healthy adults total amount of excreted protein is less than 150 mg per day; excretion of greater amounts of proteins is called proteinuria. Proteinuria can be associated with pregnancy, kidney damage, nephropathies, systemic lupus, genetic disorders, and malignancies. Elevated concentrations of urinary protein can also be caused by infections and presence of the infection-causing organism. One such disease is an invasive mycosis caused by the dimorphic fungus Histoplasma capsulatum, which results in a condition known as histoplasmosis. After spores of H. capsulatum are inhaled, the organism converts to a yeast phase, capable of disseminating through the bloodstream to various parts of the body. Disseminated histoplasmosis can become a severe and potentially fatal condition in patients with weakened immune systems (e.g., infants, elderly, severely ill; organ transplant recipients, and HIV-infected individuals). Identification of urine biomarkers associated with different causes of proteinuria may assist with differential diagnosis, elucidation of the pathogenesis of diseases, and potentially result in development of novel diagnostic tools and treatments. The goals of this study were to evaluate if differences exist among proteins excreted in urine from healthy individuals, patients with histoplasma antigenuria (HIS) and patients with proteinuria of other causes (PRU), and to determine physiological pathways associated with the proteins excreted in urine.

Materials and Methods

Patient samples
Urine samples were from 20-60 year old men. Three pools of urine samples were prepared: (i) healthy individuals, (ii) patients with proteinuria (negative for histoplasma antigen), and (iii) patients positive for histoplasma antigen (Immunomycology, Norman, OK). Each pool contained equal aliquots of urine from four individuals. The samples were tested for total urinary protein and creatinine, to account for the difference in the content of proteins in the samples from the control and the disease groups; sample volumes were adjusted to contain a total amount of protein of 0.85 mg. The study was approved by the Institutional Review Board of the University of Utah.

Methods
Urine pools were concentrated using ultracentrifugation, depletion of six abundant proteins using the MARS depletion kit (Agilent Technologies). The depleted samples were digested by trypsin using the DECU-E kit (Sigma). Purified/depleted samples were eluted from the SPE columns using solvents of increasing strength (10%, 20%, 30%, 40% and 70% of acetonitrile in water), samples were dried and reconstituted.

Materials and Methods

Sampled were analyzed on the Agilent 6510 Q-TOF equipped with a ChipCuve and series 1200 nano-HPLC system (Agilent Technologies). Peptide separation was performed on a C18 SB Zorbax Chip. Mobile phase for the analytical separation was delivered at a flow rate of 0.4 μL/min, with a gradient of 5% to 85% acetonitrile in 15 min, followed by conditioning for 2 min to initial conditions. Data acquisition was performed with the MassHunter software. The Q-TOF analyzer was tuned to a resolution of 12,000 and calibrated before each experiment for a mass accuracy of <2 ppm. The mass-dependent collision energy was used for the product ion scans. Two reference masses were added at the beginning and end of each set of samples. Peptide sequences were searched and scored using Spectrum Mill software (Agilent) Pathway analysis was performed using Ingenuity Pathway Analysis (IPA) (Ingenuity, Redwood City, CA).

Results

Controls
Medians of the total protein concentration in the urine samples of the control group, patients with PRU and HIS, were 99, 1210 and 440 μg/mL, respectively. Concentrations of the histoplasma antigen in the samples of the HIS patients ranged between 17 and 48 mg/L. Figure 1 shows total ion chromatograms (overlay of MS and MS/MS scans) of five SPE elutions of the tryptic digest of the PRU sample fraction with molecular weight 30-50 kDa and demonstrates resolution in the complexity of the data that was achieved through extensive sample fractionation. The total number of human proteins identified with high confidence in the samples was 177. 13 proteins were unique to the samples of the control group, 20 proteins were found only in the HIS group, and 20 proteins were found only in the PRU group. The distribution of proteins among the groups is shown in Table 1. Table 1 has list of proteins that were found in all samples and had over 3-fold difference in abundance among the groups. Table 2 has list of proteins, characteristic of the pathological samples which had over 3-fold difference in abundance between the PRU and HIS samples. Metabolic and cell signaling pathways, and networks of the protein-protein interaction associated with proteins identified in the urine pools are summarized in Figure 3.

Conclusions
- We identified large number of differentially expressed human proteins in the urine of PRU and HIS patients.
- In samples of PRU and HIS patients, our data suggest increase in levels of urinary proteins associated with the acute response signaling, coagulation system, anti-ganglioside presentation, and lipid antigen presentation pathways.
- Substantial differences in urinary protein profiles reflect the complexity of systemic and renal changes associated with proteinuria caused by different underlying diseases.
- Urine is a valid source of noninvasive biomarker in patients with PRU and HIS.