BIOGNOSYS
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Biognosys AG
Wagistrasse 21
8952 Schlieren (Zurich)
Switzerland

t: +41 (0) 44 738 20 40
f: +41 (0) 44 738 20 49
info@biognosys.com
www.biognosyscom

Discovery of Biomarkers in Urine of Patients with Bladder Cancer and Chronic Kidney Disease via the Investigation of Deep Proteomics

Roland Bruderer[1], Dominic G. Hoch[2], Marco Tognetti[1], Luca Raess[1], Yuehan Feng[1] and Lukas Reiter[1]

[1]Biognosys AG, Schlieren, Switzerland;
[2]Thermo Fisher Scientific, 4153 Reinach, BL, Switzerland

Introduction

Molecular biosignatures of altered proteomes in body fluids are promising in the screening and diagnosis of diseases especially those related to bladder and kidney. Investigation of urinary biomarkers are particularly attractive due to costs, time, and the noninvasive nature of urine but poses challenges due to biological and pre analytical variance. Here, we address this unmet need by applying a novel mass spectrometry-based discovery workflow which leads to unprecedented depth while maintaining high-throughput capability.

Methods

Urine samples of male patients with bladder cancer (BC, n= 27), chronic kidney disease (CKD; n= 26) and matched healthy (n= 27) were obtained from biobanks. The urine samples were processed to tryptic peptides and analyzed using a Thermo Scientific Orbitrap Exploris 480 equipped with a FAIMS Pro device. Differential abundance testing was performed in Spectronaut, and the candidate lists were filtered by an FDR <1%. Gene ontology enrichment was performed using GOrilla. Prediction models were generated in R.

Results

Using our optimized discovery proteomics workflow, we analyzed 81 urine specimens from patients with bladder cancer, chronic kidney disease and matched healthy donors. This resulted in 11,123 proteins identified in the study and 7656 proteins on average. This study reports the deepest healthy urine proteome map to date with 9645 proteins. The most significantly enriched gene ontology categories were extracellular organelles, vesicles, exosomes and cell adhesion. Unsupervised PCA clustering analysis revealed partial clustering of the conditions (already on PC1) and exploratory analysis revealed a wealth of 55 variants of post translational modifications including citrullination, hydroxylation and methylation.

First, we compared healthy to BC and found X proteins to be significantly differential abundant. We could recapitulate previously described markers like interferon alpha receptor 2 and

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urokinase plasminogen activator surface receptor, and multiple additional biomarker candidates. Next, we compared differential abundant proteins between CKD and healthy and found Y proteins differential abundant. As expected, a general elevation of protein content in urine due to CKD was detected as well as elevated levels of inflammation marker C-reactive protein, Bisphosphoglycerate mutase and High affinity copper uptake protein 1.

When comparing BC to CKD, 2158 proteins are significantly differential abundant. They are enriched in programmed cell death, telomerase organization and keratinocyte and epidermal cell differentiation. The generation of predictive models is ongoing to evaluated the classification of urine specimen by condition.

Conclusion

Harnessing the power of the latest advancement in mass spectrometry-based technique, we generated a comprehensive and quantitative map of proteomes from urine in different disease states which provided biological insights.