

In-Depth Urinary N-Glycoproteome Profiling

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OVERVIEW

Due to its simpler complexity compared with blood, urine is a desirable body fluid for diagnosis and classification of diseases, particularly of the kidney, prostate and bladder. However, an in-depth understanding of the urinary proteome remains elusive due to technical limitations. We recently developed an approach for human urinary N-glycoproteome profiling, which combined hydrazide-based N-linked glycopeptide enrichment, Off-Gel electrophoresis (OGE) fractionation, and high-performance LTQ-Orbitrap hybrid mass spectrometry.

INTRODUCTION

Fact of urine

- > A widely clinical used material for disease diagnosis and classification, especially of the prostate, bladder, and kidney;
- > Cleaner background than plasma/serum;
- > Easy to collect, big volume;
- > Less proteins derived from tissues other than kidney, prostate, and bladder;
- > Glycoprotein/peptide enriched;

Challenge of urine glycoproteome profiling

- ❖ Low total protein concentration in normal urine; <100 mg/L based on a 1.5 L/day urine output <1mg/50ml in most clinical samples
- ❖ Protein concentration strategies;
- ❖ Interference from other compounds;
- ❖ QC of collection, contaminations from blood and blood cells;
- ❖ High protein dynamic range (~10⁸)...

Previous efforts

- **Urinary proteome:**

| | | |
|------|-----------------|-------|
| 2001 | Spahr CS, et al | 124 |
| 2004 | Pieper R, et al | 150 |
| 2006 | Adachi J, et al | 1,543 |
- **Urinary glycoproteome:**

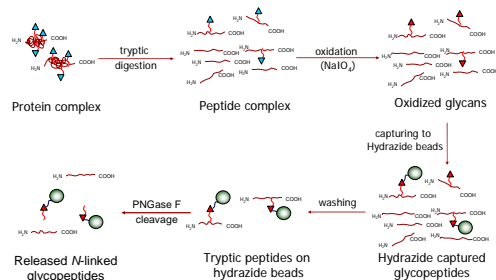
| | | |
|------|-------------------|-----------|
| 2006 | Concanavalin A | 150 |
| 2007 | Man-6-P receptors | 67 |
| 2008 | three lectins | 318 (rat) |

ACKNOWLEDGMENTS

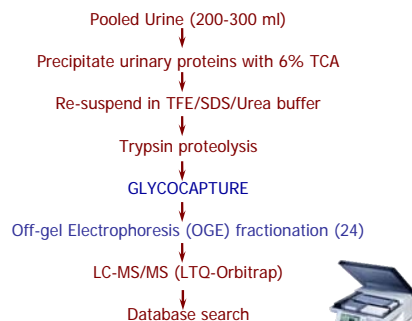
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METHODS

1. GLYCOCAPTURE METHOD [1]



2. OPTIMIZED GLYCOCAPTURE PROCEDURE FOR URINE

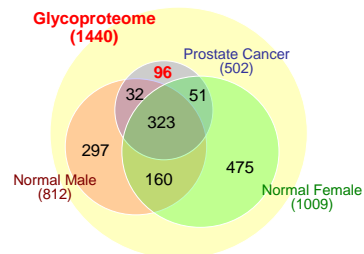


In this study, N-linked glycopeptides from three pooled urine samples (normal male, prostate cancer patient, and normal female) were enriched via the standard hydrazide-based glycopeptide capturing approach developed in our group. The enriched N-linked glycopeptides were further fractionated into 24 OGE fractions (pH3.0–10.0) and analyzed on LTQ-Orbitrap hybrid mass spectrometer.

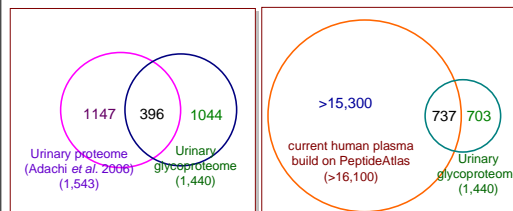
Acquired tandem MS/MS spectra were converted to mzXML and searched against the International Protein Index (IPI, version 3.38) human protein database using SEQUEST searching algorithm. PeptideProphet cut-off score of ≥0.9 (corresponding to a false-discovery rate at the peptide level of ~1.3%) was used to filter the positive identifications.

RESULTS

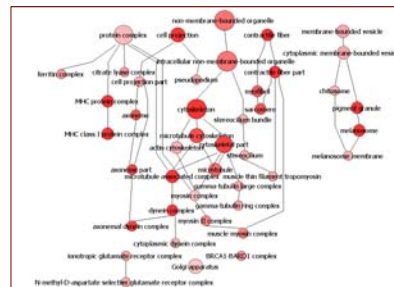
1. Distribution of identified 1,440 N-glycoproteins in three pooled urine samples collected from normal male, prostate cancer patient, and normal female



2. Overlapping of identified N-linked urinary glycoproteins with preexisting urine/plasma proteome datasets



3. Gene Ontology Cellular Component analysis of identified urine glycoproteins



4. STRING analysis of 96 glycoproteins identified only in prostate cancer patient urine



CONCLUSIONS

This work identified 1,440 distinct N-linked glycoproteins from normal and prostate cancer patient urine, the largest urinary glycoproteome dataset to date.

The relatively low overlaps for our new data with the other pre-existing urine/plasma proteome data suggests that the newly identified N-linked glycoproteins in our data are more likely to be enriched for lower abundance urine proteins. These data also indicate that the complexity and dynamic range of the urinary glycoproteome may be much higher than previously thought.

There is a significant enrichment for extracellular, cell surface, secreted, and membrane-associated proteins in our new urinary glycoproteome data.

The 96 N-linked glycoproteins which we identified only in prostate cancer patient urine give us a very good starting point to profile candidate biomarkers relevant to prostate cancer. Further evaluation on these promising findings may help to assist in building a urine-based screening method to improve the diagnostic approaches for prostate associated diseases.

REFERENCES

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