# Evaluating Proteomics in a Rat Model of Acute Kidney Injury

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## Figure 1: Proteomics Identifies Key Pathways in Acute Kidney Injury

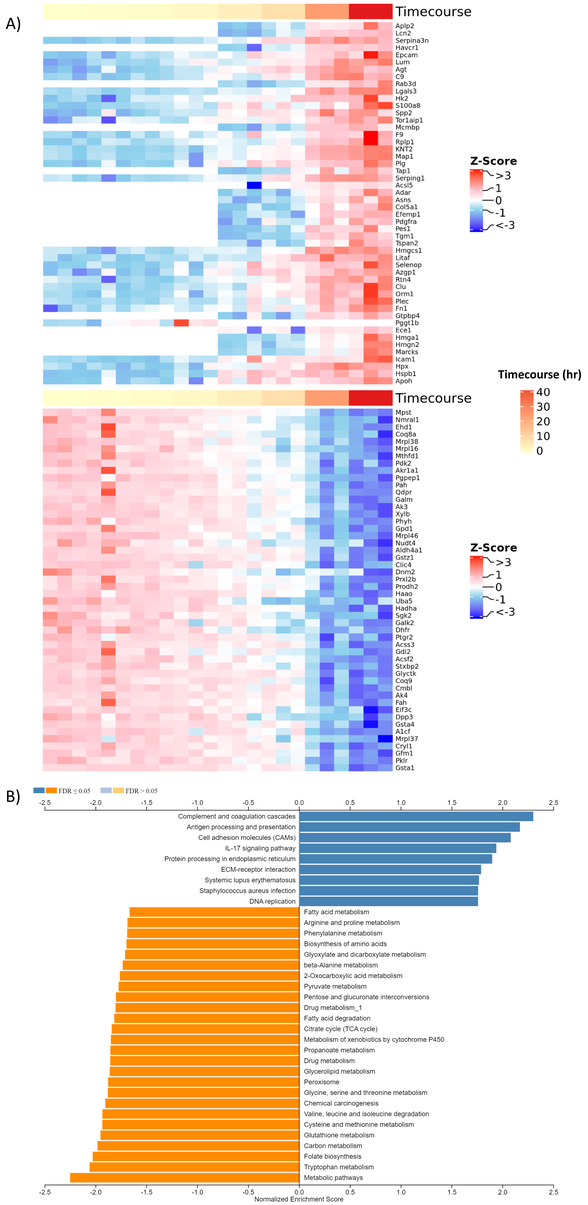


Fig 1: Proteomic heatmaps (A) show upregulated (red) and downregulated (blue) proteins across the AKI time course. (B) Pathway enrichment analysis identifies biological pathways altered by AKI over 48 hours following renal I/R.

## Figure 2: AKI Altered Expression of Kidney Injury Biomarkers and Complement Proteins

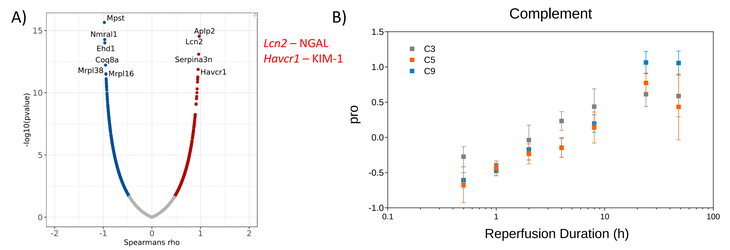


Fig 2: Proteomic data presented as a Volcano plot (A, where red=up- / blue = downregulated), and a kinetic graph (B) showing upregulation of Complement proteins across the AKI time course. Mean data +/- SEM.

## Figure 3: Gene Expression Data and Proteomics Compared Across the AKI Time Course

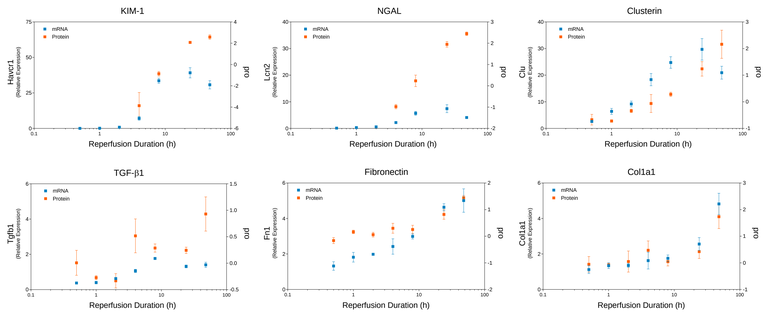


Fig 3: Gene expression (blue) and proteomic data (orange) shows temporal upregulation of kidney injury and fibrosis-associated biomarkers. Mean data +/- SEM are presented.

## Figure 4: IHC Results Show Temporal Upregulation of KIM-1 and α-SMA in AKI

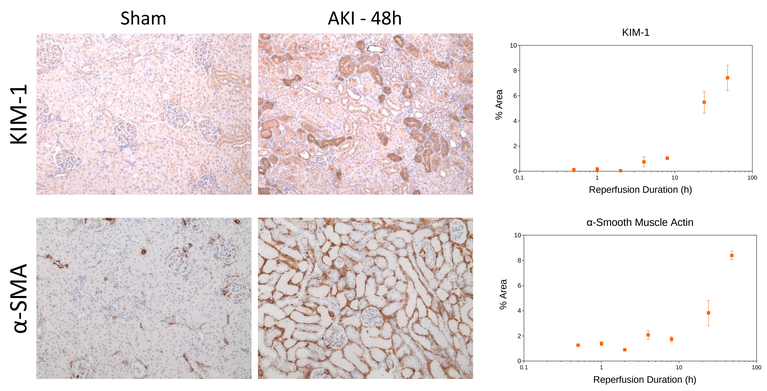


Fig 4: Immunohistochemistry (IHC) shows increased KIM-1 and α-SMA in kidney cortex/outer medulla following renal I/R. Digital image analysis shows IHC data over the entire AKI time-course. Mean data +/- SEM are presented.

## Introduction

Acute kidney injury (AKI) secondary to acute renal ischemia is associated with high mortality and morbidity, few effective treatments, and risk of chronic kidney disease. AKI therapeutics can be advanced with well characterized disease models, which inform early therapeutic development. Here we describe the proteomic landscape of a rat model of AKI and describe characteristic injury pathways and potential therapeutic targets.

## Methods

*Rat Renal I/R*: Male Wistar rats (151-175g) were housed under standard conditions, allowed food and water ad libitum acclimated for at least 1 week prior to being placed in weight-matched treatment groups. Surgical intervention was sham surgery or warm, bilateral renal ischemia (40') using a proprietary vascular occlusive device, followed by reperfusion for multiple timepoints over 48h. Over the time-course, blood and urine samples were collected and evaluated for progression of kidney injury biomarkers including BUN, creatinine, Kidney Injury Molecule (KIM-1) and NGAL. At multiple time-points, renal tissue was harvested and processed for proteomic, gene expression, and histological analyses.

*Global Proteomics*: Renal tissues were subjected to proteomic analysis using tandem mass tags (TMT) and high resolution mass spectrometry (MS) to define quantitative changes in the kidney proteome following renal I/R. Isolated kidney cortex/outer medulla tissue samples were extracted and protein digestion performed using trypsin. Tryptic digests from each sample were labeled with isobaric TMT reagents, which permits simultaneous analysis of multiple samples labeled with isotopically distinct TMT tags. Two groups of 14 samples and a reference sample were used to prepare two pools (two TMT 16-plexes) for analysis. Each pool was fractionated by high pH reverse phase separation on Thermo-Pierce spin fractionation columns to produce 8 fractions. Each fraction was analyzed by LC-MS/MS on a Thermo Fusion Lumos instrument using the Synchronous Precursor Selection method, which enables precise quantitation of the TMT tags derived from each sample in the mixture. Resultant data sets were searched against a UniProt rat proteome database with Proteome Discoverer to identify proteins, and quantitation was based on ratios of individual sample TMT tag reporter ions to reporter ions for the reference sample in each pool. Reporter ion signals for each peptide/protein were normalized to the corresponding values for the common control sample. Ratios were log2 transformed and median centered. Association between pathway/gene sets and sample attributes were computed by Gene Set Enrichment Analysis (GSEA) to identify pathways significantly altered between sham and renal I/R over the study time course.

*Quantigene Plex (QGP)*: Gene expression analysis was performed using a QuantiGene mRNA Plex Assay (ThermoFisher). Kidney (cortex/outer medulla) lysates were evaluated for expression of kidney injury and inflammatory/fibrosis-related genes including: *Havcr1* (KIM-1), *LCN2* (NGAL), *Clu* (Clusterin), *Tgfb1* (TGF-β1), *FN1* (Fibronectin), and *Col1a1* (Collagen 1), along with 3 reference genes (*Polr2a*, *Hmbs*, *Hprt*) for normalization. For each analyte, mRNA expression was normalized to the geometric mean of expression for the 3 reference genes to provide "Relative Expression" data for each analyte.

*Immunohistochemistry*: Paraffin-embedded/formalin-fixed kidney tissues were sectioned and stained using primary antibodies specific to rat KIM-1 and α-SMA. Secondary immunodetection was followed by HRP-based DAB reaction to visualize tissue localization patterns for KIM-1 and α-SMA in control and AKI tissues. Images were captured and evaluated using segmentation to calculate % Areas in control and injured kidneys for KIM-1 and α-SMA.

## Results

Proteomic analysis identified 3,084 proteins that were altered across the renal I/R time course. GSEA identified several protein networks and pathways that displayed time-dependent alteration with development of AKI. Pathways significantly upregulated with injury include complement and coagulation cascades, cell adhesion and metabolic pathways, and kidney inflammation/injury-associated pathways. Proteomic alterations correlated well with data from orthogonal approaches including gene expression profiling and immunohistochemistry.

**Examples of Altered Protein Networks & Pathways in AKI**

Upregulated:
- Complement and coagulation cascades
- Antigen processing and presenting
- Cell adhesion molecules
- Fibroblast Proliferation
- IL-17 signaling pathway
- ECM-receptor interaction

Downregulated:
- Fatty acid metabolism & degradation
- Drug metabolism
- Biosynthesis of amino acids
- Citrate cycle (TCA cycle)
- Peroxisome
- Metabolic pathways

## Conclusions

- Proteomic profiling captures both well-characterized and novel molecular alterations underlying renal injury.
- Global proteomics enables quantitative assessment of changes in the kidney proteome in the progression of AKI.
- GSEA with rich proteome data enables rapid identification of cellular pathways significantly altered following renal I/R and represents all the features of the injury phenotype.
- Global and targeted MS provides robust biomarker assays for any protein, without antibodies.
- Distinct biomarker expression profiles of varying durations of renal I/R can identify potential therapeutic targets and possible new AKI diagnostic markers.

ASN KIDNEY WEEK 2024 American Society of Nephrology October 23-27 | San Diego, CA

***Abstract #: 4128672***
***Session Title: AKI: Mechanisms [PO0103-3]***
***Poster Board #: FR-PO192***