Proteomic analysis indicates increased expression of extracellular matrix proteins in the kidneys of mice following acute kidney injury

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**Introduction**

Acute kidney injury (AKI) can result in kidney fibrosis. Common methods to assess kidney fibrosis include Picrosirius Red or Trichrome staining, and immunohistochemical staining for type 3 collagen. Proteomics – a method of measuring all protein expression levels within a sample – could be a more robust and comprehensive method of quantifying fibrosis. Proteomics by liquid chromatography tandem mass spectrometry (LC-MS/MS) offers thorough analysis into changes to the kidney extracellular matrix (ECM), but previous studies fail to capture the ECM proteome in depth. Here, following an ECM-targeted extraction protocol, we performed LC-MS/MS on AKI vs. sham kidneys in mice to determine changes to the ECM following AKI. The overall goal is to identify therapeutic targets for AKI.

**Methods**

- All animal studies were approved by the UC IACUC.
- Adult (10-14 weeks old) C57BL/6J male mice survived a standardized, bi-lateral ischemia re-perfusion acute kidney injury (AKI). Sham mice received surgery but no AKI\(^1\).
- At 3 months following AKI, animals were euthanized by CO\(_2\)-asphyxiation, kidneys were harvested, processed for ECM extraction, and were subjected to liquid-chromatography coupled with tandem mass spectrometry (LC-MS/MS).\(^2\)
- Resulting mass spectra were processed by label free quantification (PD: Mascot 2.5.1; Sieve 2.1).
- Intensity values of identified proteins were compared across timepoints. Proteins were determined to have statistical difference by ANOVA or t-test with FDR-adjusted p-values < 0.05.
- Differentially expressed proteins (DEPs) were determined to have FDR p-values < 0.05 with > 2x fold change difference in expression between timepoints.

**Results**

In total, 92 kidney ECM proteins were identified using LC-MS/MS. Hierarchical clustering and principal component analysis (not shown) demonstrated clustering of AKI and sham kidneys by their ECM proteomes (A), indicating global differences in these proteomes. Proteins expressed at high levels in AKI relative to sham include PRELP, BGN, ECM1, LUM, MATN2, and OGN which all contribute to ECM anchoring; collagen, fibrillar, or filament assembly; or ectopic calcification (B), indicating increased ECM production following AKI. Enrichment resulted in ‘ECM organization’ as the top term.

Proteins expressed at low levels in AKI kidneys include TGM1, ITIH5, ADAM10, and NTN4, which are involved in proteinolyis and ECM stabilization (B), suggesting reduced tissue maturation and remodeling following the AKI wound. Strong correlations between AKI and the ECM proteins COL1A1, COL1A2, COL3, TNC, and FGG were also identified (C). MATN2 and TNC (involved in ECM and tissue formation) were identified as top proteins contributing to AKI injury status in random forest classification (D).

**Conclusion**

Our data suggest an increase in tissue ECM production, especially fibrillar collagens and ECM assembly 3 months after AKI, findings which are corroborated throughout the literature. Future studies will investigate different timepoints, changes in cell response (qPCR), and mining these data for differences in other hallmark proteins involved in fibrosis and in the progression of AKI.

This approach using LC-MS/MS could be used for proteomic investigation and characterization of the kidney ECM following AKI, which could lead to the identification of key changes to the ECM architecture (therapeutic targets) following AKI.

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