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A CLASSICAL AND AN IMMUNOAFFINITY-PROTEOMIC STUDY TO IDENTIFY AND VALIDATE DRUG-INDUCED KIDNEY INJURY BIOMARKER IN CANINE

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OVERVEIW

- Drug-induced kidney injury in canines was investigated at the proteome level
- Male beagle canines received the nephrotoxic dose of tobramycin (60 mg/kg, n=6) or vehicle (n=3) for ten days
- Kidney injury was confirmed by routine histopathology
- Proteome changes were investigated by label-free quantification analysis of the fresh frozen kidney tissue samples in response to tobramycin nephrotoxicity (tech rep n=3)
- 558 proteins were significantly dysregulated (q-value < 0.05) between the two groups
- Multiplex immunoaffinity-Mass Spectrometry (IP-LC/ t(MS)) assay was used to precisely quantify the biomarker candidates in the tissue and urine canine samples (Table 1)
- A significant increases in urinary clusterin, urinary retinol binding protein 4, urinary kidney injury molecule-1 and urinary alpha-1-macroglobulin were detected on study day 10
- This study revealed the utility of candidate biomarkers for identifying tobramycin-induced kidney injury in dogs
- Our signature epitope antibodies comprise epitopes conserved in monkey, rat, mouse and human. Hence enrichment strategy can be applied across different species.

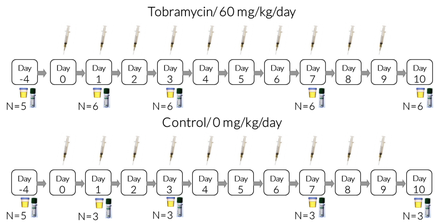
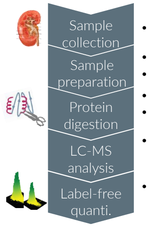


Table 1: List of drug induced kidney injury protein candidates investigated in this study. (+) up-regulated proteins in treated dogs (*p*-value < 0.05), (-) no significant change observed, (*) immunoaffinity-MS assay not yet established in urine.

Protein	Label-free analysis (Tissue)	IP-LC/MS (Tissue)	IP-LC/MS (Urine)
alpha-1-macroglobulin (AMBP)	+	+	+
Clusterin (CLU)	+	+	+
Retinol Binding Protein 4 (RBP4)	+	+	+
Kidney Injury Molecule -1 (KIM-1)	Not detected	+	+
Osteopontin (SPP1)	-	+	+
Cystatin-C (CST3)	+	+	-
Serpin family A member 5 (SERPINA5)	+	+	*
Poly(rC) binding protein 1 (PCBP1)	+	*	*
Zinc-alpha-2-glycoprotein (AZGP1)	+	*	*
Fatty acid-binding protein (FABP)	+	-	*
Aquaporin 2 (AQP2)	Not detected	-	-
Podocin (NHPS1)	Not detected	-	-

WORKFLOW

Discovery study Label-free quantification

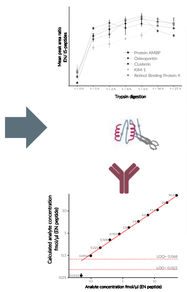


- Kidneys were collected at day 10
- Lyse tissue in ball mill
- Protein concentration
- O/N in-gel digestion
- Peptide conc. via NanoDrop
- DDA analysis on Q Exactive Plus™ MS connected to UPLC
- Data analysis via MaxQuant

Verification analysis in tissue

- Candidates selection
- In silico digest/ peptide selection
- Antibody Generation
- Synthesis of standard peptides
- Assay optimization
- O/N insolution digestion
- Enrich peptides with signature epitope
- Automated enrichment
- Multiplex targeted analysis (PRM)
- Endogenous : SIS ratio for quant

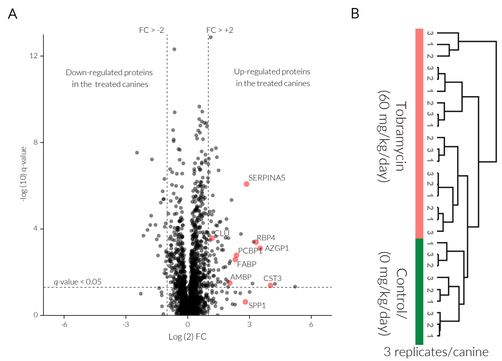
Verification analysis in Urine



- Digestion kinetics
- Intra-day variation
- Inter-day variation
- Thaw cycle
- Assay linearity
- 2 hr urine digestion
- Enrich peptides with signature epitope
- Automated enrichment
- Multiplex targeted analysis (PRM)
- Quantification based on SIS

RESULTS

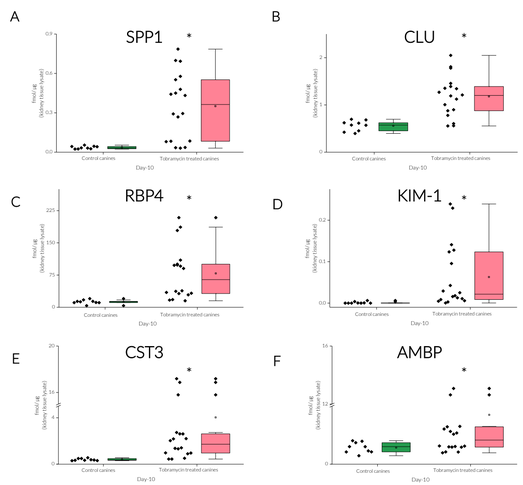
Label-free tissue-based quantification analysis



A) Volcano plot demonstrating the label-free discovery tissue analysis.
- 2041 proteins were identified and quantified from the LF analysis
- 558 proteins were differentially sig. regulated (q-value < 0.05)
- 84 proteins were up-regulated in the treated dogs (2 FC, q-value <0.05
- The highlighted dots represent the selected candidates for the multiplex IP-LC/ t(MS) verification analysis

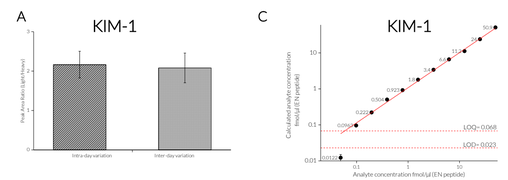
B) Unsupervised hierarchical clustering of treated and controls canines
- Based on protein expression values the samples were clustered into distinct two distinct groups (treated and controls canines).

Verification analysis in kidney tissue using Multiplex IP-LC/ t(MS)



A-F) Relative quantification of drug induced kidney injury biomarker candidates in kidney tissue samples at day 10
- The investigated candidates were found to be significantly up-regulated in association with induced kidney injury (* *p*-value < 0.05).

Verification analysis in urine using multiplex IP-LC/ t(MS)



Representative results obtained from KIM-1 assay development of in urine:

A) Assay reproducibility assessment

B) Regression curve IP-LC/ t(MS) of KIM-1 targeted peptide

C) Absolute urinary KIM-1 concentration in tobramycin treated-dogs and controls

Similar protein expression profiles were also obtained for urinary CLU, AMBP SPP1 and RBP4.

CONCLUSION

We presented a differential proteome analysis comparing kidney tissue samples collected tobramycin-treated canines and controls (see also Abstract #1252/ Poster board # P349). A multiplex immunoaffinity-MS assay was developed to quantify drug-induced kidney injury marker from canine tissue and urine. We confirmed potential biomarker for DIKI in tissue and urine.
Because of signature epitope antibody enrichment strategy this assay can be further expanded to cover monkey, rat, mouse and human specimen.