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P 631

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A classical and an immunoaffity-proteomic study to identify and validate drug-induced kidney injury biomarker in Canine

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OVERVIEW

- Drug-induced kidney injury in canines was investigated at the proteome level
- Male beagle canines received a 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 fresh frozen kidney tissue samples in response to tobramycin nephrotoxicity (technical replicates n=3)
- 558 proteins were significantly dysregulated (q-value < 0.05) between the two groups
- Multiplex Immunoaffinity-Mass Spectrometry (IP-LC/ tMS) assay was used to precisely quantify the biomarker candidates in the tissue and urine canine samples (Table 1)
- Increases in osteopontin, clusterin, retinol binding protein 4, kidney injury molecule-1 and alpha-1-macroglobulin were detected in the urine on study days 7 and 10
- This study revealed the utility of candidate biomarkers for identifying tobramycin-induced kidney injury in dogs
- Our Triple X Proteomics (TXP) antibodies comprise epitopes conserved in monkey, rat, mouse and human. Hence enrichment strategy can be applied across different species.

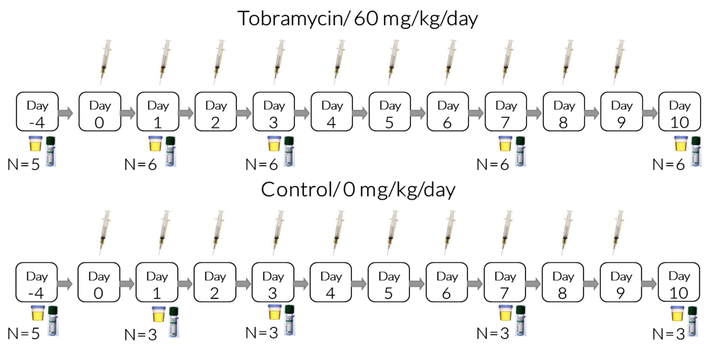
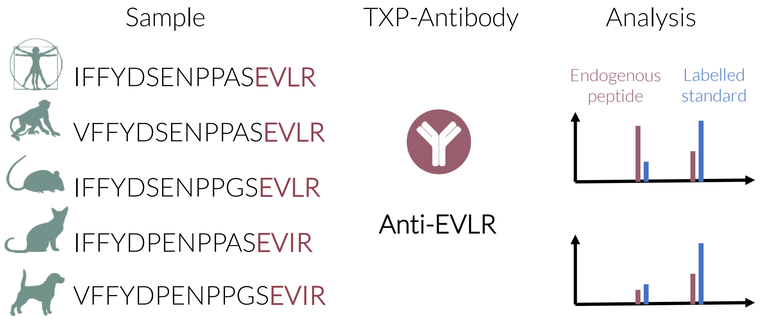


Table 1: List of drug induced kidney injury protein candidates investigated in this study. (+) up-regulated proteins in treated dogs (*p*-value < 0.05), (-) no significant change observed, (*) immunoaffinity-MS assay not yet established in urine.

Protein	Label-free Analysis (Tissue)	IP-LC/MS (Tissue)	IP-LC/MS (Urine)
Alpha-1-microglobulin (AMPB)	+	+	+
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	-	-

CROSS-SPECIES IMMUNOASSAYS



SUMMARY

We confirmed biomarkers for drug-induced kidney injury in tissue and urine samples from tobramycin-treated canines and controls using MS-based Immunoassays. A multiplex immunoaffinity-MS assay was developed to quantify drug-induced kidney injury markers from urine and tissue specimens which can be used for non-human primates, rodents, canines, felines and humans.

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WORKFLOW

Biomarker discovery phase

Mass spectrometry-based differential proteome analysis "classical approach"

- Dogs treated with Tobramycin
- Kidneys collected at day 10
- Tissue homogenized in ball mill
- O/N in-gel digestion using Trypsin
- Data dependent acqisition (DDA)

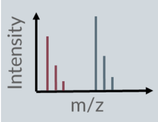
LC-MSMS

Label free quantification via MaxQuant

Biomarker verification phase/ tissue-based

Biomarker verification in tissue immunoaffinity-MS-based approach

- Sample: 20 µg tissue extract / 50 µl urine
- Digestion using Trypsin
- Adding isotope-labelled peptide standard
- Enrich endogenous and standard peptides
- Multiplex targeted nLC-MSMS-based analysis (PRM)

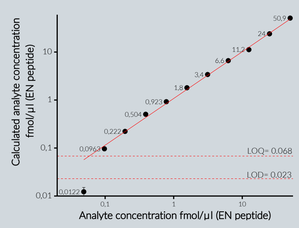


Quantification by internal isotope-labeled standards

Assay development / Urinary biomarker verification phase

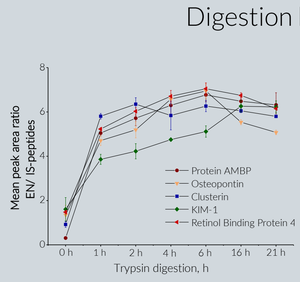
Immunoaffinity MS-assay development & Biomarker verification in urine

AB functionality and assay linearity



- Regression curve IP-LC/ MS of KIM-1 targeted peptide
- Assay performance is comparable for CLU, AMBP, SPP1, NPHS2, AQP2 and RBP4
- Red doted lines represent the LOD and LOQ of KIM- 1 assay

Digestion kinetics



Release kinetics profile of targeted peptides from urinary CLU, AMBP, SPP1, RBP4 and KIM-1 in canines

- The digestion kinetics was monitored over 21h
- 2h urine digestion was optimized for the optimal multiplex assay

Inter/ Intra-day variation

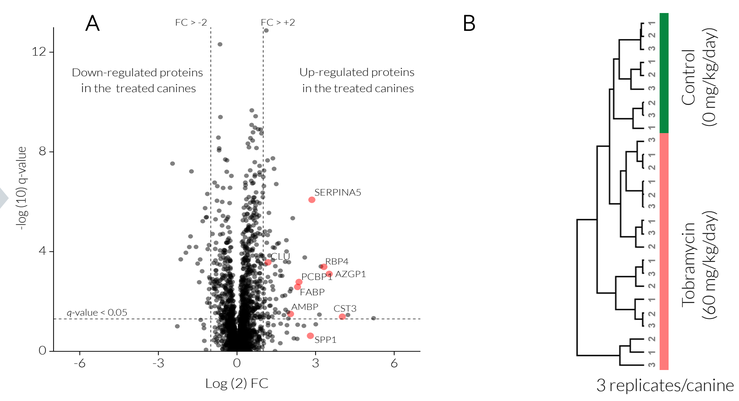
Representative results obtained from urinary KIM-1 assay development in canine urine

- Intra-day var n=8 (CV =15 %)
- Inter-day var n=24 (CV =18 %)

Quantification by internal isotope-labeled standards and external standard curve

RESULTS

Differential proteome analysis in kidneys from Tobramycin-treated canines by label-free quantification

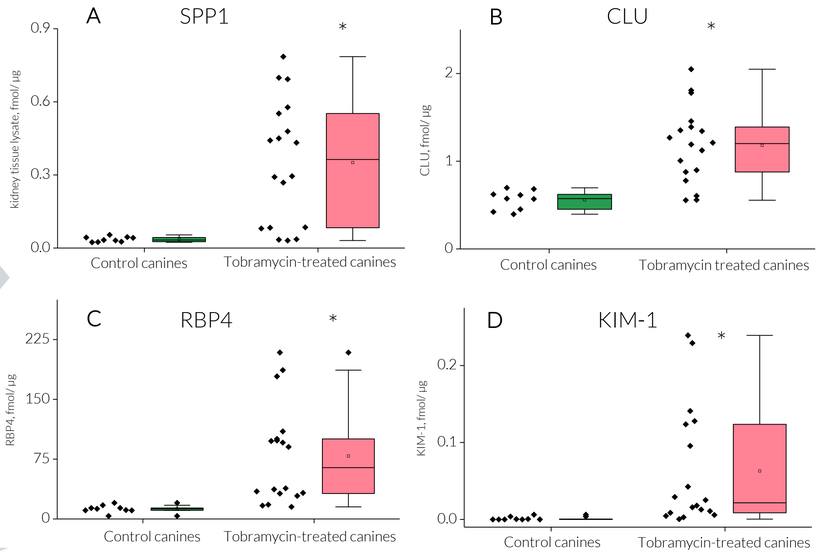


A)Volcano plot showing the label-free discovery tissue analysis

- 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

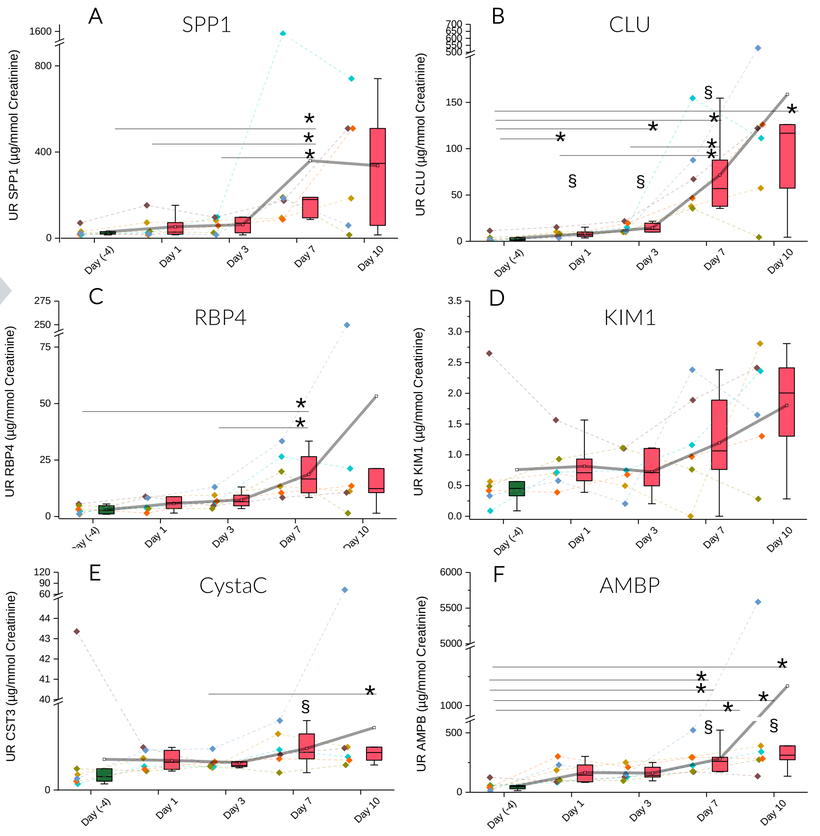
B) Protein expression profiles were clearly separated into Tobramycin-treated and control groups by unsupervised hierarchical clustering.

Biomarker verification in kidney tissue using Multiplex IP-LC/ tMS



A-D) Relative quantification of drug induced kidney injury biomarker candidates in kidney tissue samples at day 10. These parameters were found to be significantly up-regulated in association with induced kidney injury (* p-value < 0.05). Data for CST3 and AMBP are not shown

Urinary biomarkers of kidney toxicity (multiplex IP-LC/MS assay)



A-F) Absolute quantification of drug induced kidney injury biomarker candidates in urine samples from tobramycin-treated dogs. When normalized to Ur creatinine, multiplex IP-LC/MS assay revealed increases in Ur clusterin, Ur osteopontin, Ur AMBP, Ur RBP4 and Ur KIM-1 on Day 7 and D10 in tobramycin-treated dogs vs controls (* p<0.05, Wilcoxon Signed Ranks Test)