Urine Proteomics for Liquid Biopsy of the Kidney

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Objectives:

- **Diagnosis and assessment of severity of kidney diseases are often examined by kidney biopsy. However, the kidney biopsy is invasive and the patients need to be hospitalized. Therefore, the procedure is not always applicable frequently.**
- **Recently omics such as genomics, transcriptomics, proteomics and metabolomics has been introduced in nephrology to understand pathophysiology of human diseases by comprehensive analysis of genes, transcripts, proteins or metabolites and prediction of molecular events such as pathways in the tissues.**
- **The liquid biopsy is A clinical application of the omics, which has been introduced to detect a disease, such as a cancer and further more to make differential diagnosis possible by examining biofluid such as blood without information of pathological changes obtained by biopsy.**
- **We aimed to utilize proteomics approach to discover biomarkers in urine, which inform physiologic or pathologic conditions in the kidney, and to select a urine biomaker panel, which may substitute for the kidney biopsy as urine liquid biopsy.**

Methods:

STEP1 Preparation of Tryptic peptides: **Tryptic peptide preparation from parts of human kidney; glomerulus, proximal tubule, distal tubule, collecting duct, cortex, or medulla, by laser microdissection of formalin-fixed and paraffin-embedded (FFPE) human kidney tissue sections combining with immnohistochemisty using antibodies to nephron-unique proteins, followed by direct digestion of each kidney section with trypsin (On-Site Direct Digestion, OSDD).**

STEP2 Mass spectrometry for protein identification and semi-quantification of proteins: **Peptides were separated by a reversed-phase liquid chromatography using a C-18 column and analyzed by a tripleTOFmass spectrometer (5600+, SCIEX) (LC-MS). Proteins were identified by Mascot search engine (Matrix Science) using the Human Proteome Database (Uniprot) and semi-quanititated by modifed Spectrum Index normalized (SI_N).**

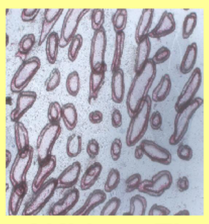
STEP3 Urine and plasma proteomes: **Urine proteins were prepared from healthy volunteers and separated by SDS-PAGE for in-gel gigestion with trypsin for LC-MS as the kidney tissue peptides. Plasma proteomes were obtained from the human plasma proteome datasets (HPPP).**

STEP4 Selection of kidney-site-specific urine biomarker proteins: **By comparing all the kidney tissue part proteomes and urine and plasma proteomes, kidney-site-specific protein biomarker candidates were selected. Their localization in the kidney was further validated by referring the Human Protein Atlas (HPA) database and by our immunohistochemistry.**

STEP5 Antibody-based trial measurement and comparison with kidney biopsy for Liquid Biopsy: **The amounts of these kidney-site-specific urine proteins in urine samples from healthy volunteered and kidney disease patients were measured by surface plasmon resonance (ProteOn, BioRad) using antibodies for the proteins. The amounts and histological evaluation of kidney baipsies were compared to know whether the amounts might correlate with the histological severity of kidney tissue parts.**

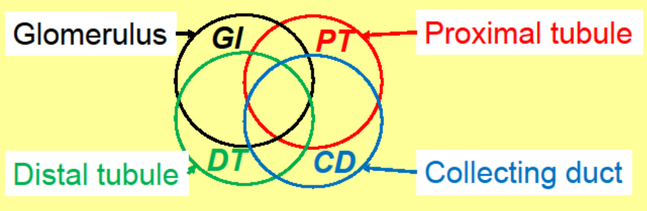
Results:

STEP1 Microdissection of Kidney Tissue Parts

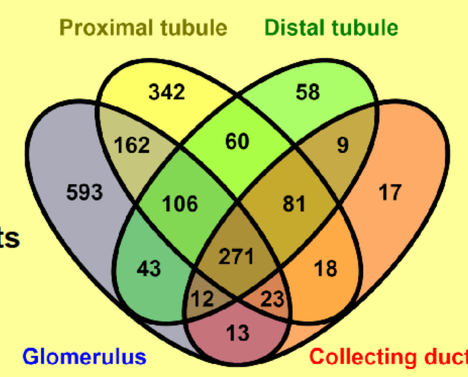


STEP2 MS Proteome Analysis of Kidney Tissue Parts

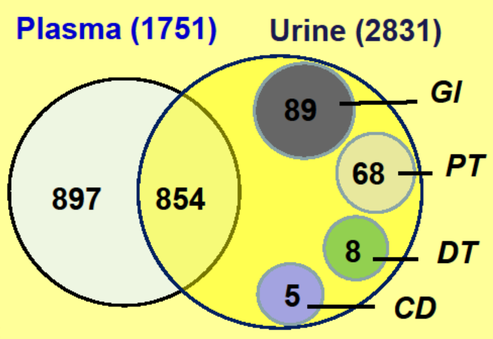
	Glomerulus	Prox tubule	Dist tubule	Collect duct	Urine	Plasma
# Datasets	5	7	4	5	10	HPPP (Ref.)
# Gene symbols	1223	1063	640	444	2831	1751



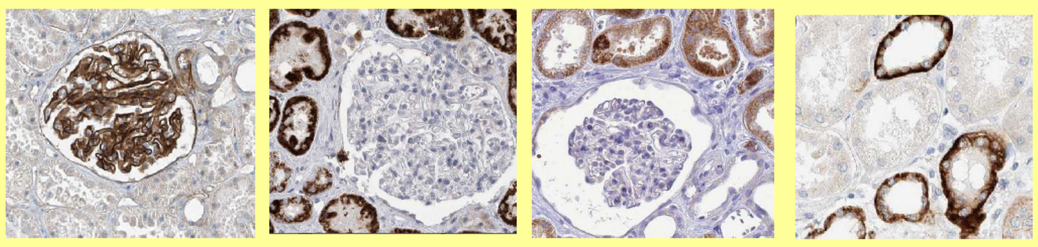
STEP2



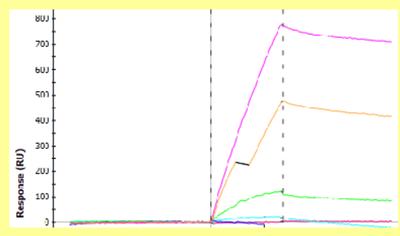
STEP3 and 4

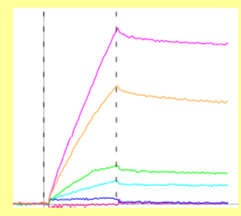


STEP4 Validation by Immunohistochemistry



STEP5 Urine Excretion of kidney-site-specific proteins examined by Surface Plasmon Resonance(SPR) using mAbs





CKD:
Severe
Severe
Moderate
Mild injury

Control

Conclusions:

By analyzing urine biomarkers for kidney-site-specific injuries, kidney histological lesions may be visualized as liquid biopsy in quantitative and qualitative manners. The liquid biopsy will beneficial for sequential evaluation of kidney tissue changes in stead of sequential biopsies.

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