TITLE

MASS SPECTROMETRY- AND ANTIBODY-BASED PROTEOMICS OF THE HUMAN KIDNEY AND URI

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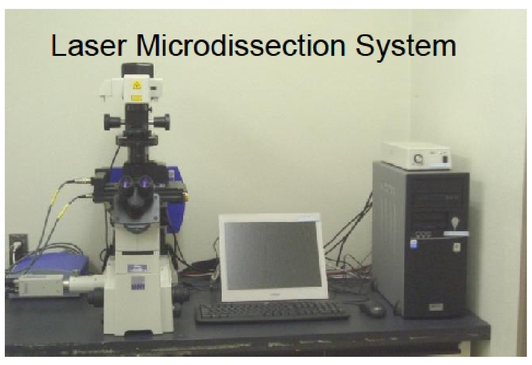
OBJECTIVES

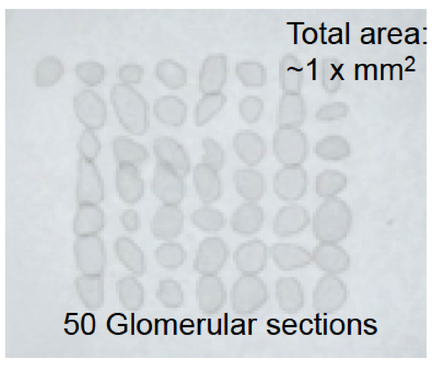
Functions of the kidney and nephron segments are obituary known, however, the precise details have not been clarified yet. Pathophysiological mechanisms of human kidney diseases have also not been disclosed. Proteomics is a powerful tool to analyze proteomes of kidney, nephrons or urine for understanding functions, protein interactions (pathways), pathophysiology and biomarker discovery.

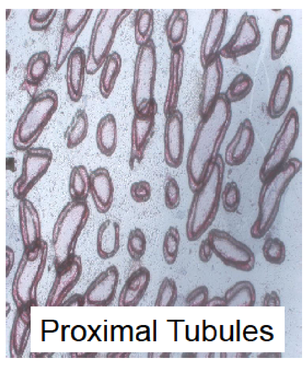
METHODS

Normal parts of tissues (cortex, medulla and glomerulus) were obtained from kidneys nephrectomized due to renal cancers. Urine samples were also collect from healthy volunteers. Tissue and urine proteins were separated by gel electrophoresis and peptides were prepared by in-gel trypsin digestion for mass spectrometry (MS).
Sections of nephron segments were obtained by laser-microdissection from nor human kidney sections, which were immunostained with anti-AQP1, calbindin a AQP2 antibodies, for identification of each nephron segment (proximal, distal tu and collecting duct, respectively). Glomerular sections were also collected from kidney biopsy samples of kidney disease patients (membranous nephropathy, Ig nephropathy and others). The peptides were prepared by direct digestion of the sections with trypsin (On-Site Direct Digestion) method for MS.
Antibody (Ab)-based analysis of human tissues have been carried out in the Hu Protein Atlas (HPA) project and more than a half of human proteins have been localized in the human body and also in the kidney by immunohistochemistry (IH

Laser Microdissection







Proteins identified in the Glomerulus of Normal (Nx) and Membaranous nephropathy (MN)

Sample name	Original disease	Raw file	MQ ID			GO ID (human)	DAVID ID
NX020902 (Glo-3)	Kidney cancer	glomeruli _sample03	16,357	IPI ID	7,084	4,531	1,607
				UNIPROT ID	9,273	1,685	1,614
NX020902 (Glo-4)	Kidney cancer	glomeruli _sample04	17,591	IPI ID	7,010	4,606	1,664
				UNIPROT ID	10,581	1,706	1,656
NX020902 (Glo-5)	Kidney cancer	glomeruli _sample05	17,198	IPI ID	6,924	4,545	1,640
				UNIPROT ID	10,274	1,702	1,634
MN_A876-1	MN	glomeruli 02	13,799	IPI ID	5,188	3,361	1,226
				UNIPROT ID	8,611	1,293	1,228
MN_H101672-1	MN	glomeruli 03	18,942	IPI ID	7,871	5,089	1,833
				UNIPROT ID	11,071	1,925	1,847
MN_H091546-1	MN*	glomeruli 06	16,142	IPI ID	6,745	4,289	1,591
				UNIPROT ID	9,397	1,663	1,590
MN_H110928-1	MN	glomeruli 07	19,343	IPI ID	8,147	5,248	1,876
				UNIPROT ID	11,196	1,946	1,885

	Protrein	GO: Molecular Functions
Glom	1325	structural molecule
		actin binding
		structural cytoskeleton
Proximal Tubule	1940	oxidoreductase activity
		catalytic activity
		cofactor binding
Distal Tubule	1179	structural molecule
		nucleotide binding
Collecting Duct	1051	GTP binding
		guanyl nucleotide binding
Urine	1189	

Gene Ontology Annotation of Nephron Proteomes

RESULTS

MS identified more than a thousand proteins with hi confidence in each component of the normal human kidney. The Ab-based proteomics disclosed thousan of proteins in the kidneys. Comparison of the MS-ba and Ab-based glomerulus proteomes showed approximately one fourth of proteins, which were identified by MS or Ab, were detected by both MS- a Ab-based methods. About a half of urine proteins identified by MS were also found in human plasma proteomes. Urine proteins, which were not plasma proteins, were localized in the kidney and other urin tract by looking at the HPA IHC images. The localiza of urine proteins were summarized in a human urine proteome database. By MS analysis of human glomerular sections of each kidney biopsy samples, approximately a thousand proteins were identified a were further analyzed by bioinformatics to understa pathophysiology of kidney diseases.

CONCLUSIONS

Proteomic analysis of human kidney tissues and urine provided function- and disease-related information. Bioinformatics and validation of these data are expected to disclose the kidney functions and pathophysiology of kidney diseases for precise mechanisms of the progression or pathogenesis. Urine proteomics may elucidate biomarker candidates for detection of injury of each nephron segment.

References

Human Proteome Organisation:
http://www.hupo.org/
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