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A Comprehensive Map of the Human Urinary Proteome

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Abstract

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ASSOCIATED CONTENT

Supporting Information

Supplementary Table 1, Details of samples used in this study. Supplementary Table 2, List of all the peptides (2A) peptides from unfractionated urine (2B) and peptides from lectin enriched fraction (2C). Supplementary Table 3, Complete list of proteins identified from this study. Supplementary Table 4, Complete list of proteins identified by lectin affinity enrichment. Supplementary Table 5, List of proteins identified in common to the three large scale studies on urinary proteome. Supplementary Table 6, Proteins identified in common to this study and urinary exosomal proteins reported by Pisitkun et al., 2004 and Gonzales et al., 2009. Supplementary Figures: MS/MS spectra for a subset of proteins identified based on single peptide. This material is available free of charge via the Internet at http://pubs.acs.org.

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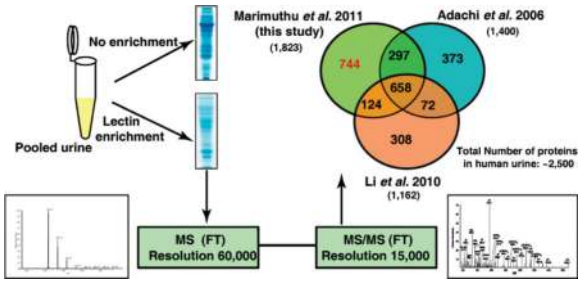
The study of the human urinary proteome has the potential to offer significant insights into normal physiology as well as disease pathology. The information obtained from such studies could be applied to the diagnosis of various diseases. The high sensitivity, resolution, and mass accuracy of the latest generation of mass spectrometers provides an opportunity to accurately catalog the proteins present in human urine, including those present at low levels. To this end, we carried out a comprehensive analysis of human urinary proteome from healthy individuals using high-resolution Fourier transform mass spectrometry. Importantly, we used the Orbitrap for detecting ions in both MS (resolution 60 000) and MS/MS (resolution 15 000) modes. To increase the depth of our analysis, we characterized both unfractionated as well as lectin-enriched proteins in our experiments. In all, we identified 1823 proteins with less than 1% false discovery rate, of which 671 proteins have not previously been reported as constituents of human urine. This data set should serve as a comprehensive reference list for future studies aimed at identification and characterization of urinary biomarkers for various diseases.



Keywords

body fluids; biomarkers; glycoproteins

INTRODUCTION

Urine is formed through filtration of plasma by glomeruli in the kidneys. The glomeruli act as a filter to retain most of the proteins.[1–3] Ultimately, the excreted urine contains water, glucose, salt and other metabolites in addition to small amounts of proteins, which can be derived from the serum proteins or from the kidney itself.[3] From a proteomic standpoint, the urine therefore can provide readout of the systemic physiology (from serum proteins) as well as local physiology (from the proteins contributed by kidneys).

Several studies have been carried out in the past employing different approaches to characterize the urinary proteome.[4, 5] One of the earliest studies to characterize the urinary proteome employed two-dimensional gel-based electrophoresis and reported 250 spots.[6] Decades after this initial study, another group observed 1400 spots in urine employing two-dimensional gel electrophoresis but could identify only 30% of the proteins observed in the gel.[7] In subsequent studies, two-dimensional gel electrophoresis followed by MALDI-TOF mass spectrometry resulted in the identification of 120–150 proteins in urine.[8, 9] Subsequently, studies employing LC–MS/MS have contributed to a significant increase in the number of identified proteins in urine.[10–14] However, the aforementioned studies were still limited by the sensitivity of the mass spectrometers used. The study by Adachi et al.,[15]

and a recent study by Li et al.[16] where they have used LTQ-Orbitrap mass spectrometer, led to the identification of more than 1000 proteins in urine. In both of these studies, although the MS detection was carried out at high resolution in the Orbitrap, the MS/MS detection was performed at a much lower resolution in the linear ion-trap.

From these studies, it is clear that we do not yet have a comprehensive view of the urinary proteome. It is important to have a good baseline reference proteome because urine can serve as an excellent body fluid for identification of biomarkers for several reasons. First, collection of urine is a noninvasive procedure. Second, the composition of urine is less complex as compared to serum or plasma, which makes it more attractive for mass spectrometric analysis.[1, 17] A number of studies have been carried out to identify urinary markers in various disease conditions and candidate biomarkers have been identified for several kidney related disorders including diabetic nephropathy,[18, 19] acute renal injury and obstructive nephropathy.[17] In addition to these renal disorders, potential biomarkers for systemic illnesses have also been identified from urine including prostate cancer,[20] bladder cancer[21] and colon cancer.[22]

In this study, we report the largest catalog of proteins in urine identified in a single study to date. This was achieved by an in-depth analysis using high-resolution Fourier transform mass spectrometry using high-resolution settings at both MS and MS/MS levels. The analysis was carried using samples pooled from healthy individuals. The use of pooled sample is still arguable, as the normal urine concentration varies from one individual to another.[23] However, in this study, we decided to pool urine from multiple individuals to account for the heterogeneity and to obtain a comprehensive coverage of urinary proteome from healthy subjects. This study is an attempt to discover the maximum number of identifiable proteins in urine. We report 1823 proteins in human urine of which 671 proteins have not been previously reported in urine.

MATERIALS AND METHODS

Sample Collection and Processing

Twenty four healthy individuals were recruited for the study. Details of the samples used in this study are provided in Supplementary Table 1 (Supporting Information). Informed consent was obtained from all subjects and the study was approved by IRB. Thirty to 50 mL of urine was collected from each individual and stored on ice prior to processing. The samples were centrifuged at 1500× *g* for 10 min at room temperature. The cleared supernatant was further filtered using 0.22 μm filter (Millipore, Billerica, MA). Twenty milliliters of filtered urine from each individual was concentrated using 3 kDa cutoff filters (Millipore, Billerica, MA). The concentrated urine, that is, the retentate, was stored immediately at −80 °C until further use. Equal volumes of concentrated urine samples from the 24 individuals were pooled and protein concentration was determined using Lowry's assay.

Sample Fractionation

Approximately 250 μg of protein from pooled urine sample was resolved by ten percent SDS-PAGE (16 × 18 cm gel). The gel was stained with colloidal coomassie blue (Invitrogen, Carlsbad, CA). Thirty gel bands were excised, destained and subjected to in-gel digestion as previously described.[24] Briefly, the excised bands were reduced with 5 mM dithiothreitol in 40 mM ammonium biocarbonate followed by alkylation with 10 mM iodoacetamide in 40 mM ammonium bicarbonate. Digestion was carried out using trypsin (modified sequencing grade; Promega, Madison, WI) at 37 °C for 16 h. The peptides were extracted from the gel slices as described previously,[24] dried and stored at −80 °C until LC–MS/MS analysis. The extract from each gel slice was split in to two and analyzed separately as two independent runs.

Lectin Affinity Enrichment

Twelve milligrams of total protein was diluted in 10 mM phosphate buffer pH 7.8. For glycoprotein enrichment, the samples were incubated with a mixture of three agarose conjugated lectins—concanavalin A, wheat germ agglutinin and jacalin (Amersham BioSciences)—for 12 h at 4 °C. The beads were then washed three times using wash buffer (10 mM phosphate buffer pH 7.8) and the bound proteins were eluted using a mixture of carbohydrates (100 mM each of *N*-acetylglu-cosamine, melibiose and galactose). The eluate was dialyzed against wash buffer to remove free sugars and concentrated using 3 kDa cutoff filters. The concentrated proteins were measured by Lowry's method. One fourth (500 μg from 12 mg) of the proteins were found to be enriched using lectin affinity chromatography. Half the amount of the concentrated protein (250 μg) was then resolved by SDS-PAGE and visualized using colloidal coomassie staining. Twenty-four bands were excised and subjected to in-gel trypsin digestion procedure as previously described.[24] The extract from each gel slice was split in to two and analyzed separately as two independent runs.

LC–MS/MS analysis

Nanoflow electrospray ionization tandem mass spectrometric analysis of peptide samples was carried out using LTQ-Orbitrap Velos (Thermo Scientific, Bremen, Germany) interfaced with Agilent's 1200 Series nanoflow LC system. The chromatographic capillary columns used were packed with Magic C_{18} AQ (Michrom Bioresources, 5 μm particle size, pore size 100 Å) reversed phase material in 100% acetonitrile at a pressure of 1000 psi. The peptide sample from each fractionation method was enriched using a trap column (75 μm × 2 cm) at a flow rate of 5 μL/min and separated on an analytical column (75 μm × 10 cm) at a flow rate of 300 nL/min. The peptides were eluted using a linear gradient of 7–30% acetonitrile over 50 min. Mass spectrometry analysis was carried out in a data dependent manner with full scans acquired using orbitrap mass analyzer at a mass resolution of 60 000 at 400 *m/z*. For each cycle, twenty most intense precursor ions from a survey scan were selected for MS/MS and detected at a mass resolution of 15 000 at *m/z* 400. The fragmentation was carried out using higher-energy collision dissociation as the activation method with 40% normalized collision energy. The ions selected for fragmentation were excluded for 30 s. The automatic gain control for full FT MS was set to 1 million ions and for FT MS/MS was set to 0.1 million ions with a maximum time of accumulation of 750 and

100 ms, respectively. For accurate mass measurements, the lock mass option was enabled. Internal calibration was carried out using the Polydimethylcyclosiloxane (*m/z*, 445.1200025) ions.

Data Analysis

The mass spectrometry data was processed using Proteome Discoverer (Version 1.2.0.208) software (Thermo Fisher Scientific) workflow and searched using Mascot and Sequest as search nodes with Xtract feature enabled against NCBI RefSeq release 40 human protein database containing 31 811 sequences. The search parameters used were oxidation of methionine, deamidation at N and Q and protein N-terminal acetylation as variable modifications and carbamidomethylation of cysteine residues as a fixed modification. A maximum of one missed cleavage was allowed for tryptic peptides. The peptide and protein data were extracted using high peptide confidence and top one peptide rank filters. False discovery rate was calculated by enabling the peptide sequence analysis using decoy database. Mass error window of 20 ppm and 0.1 Da were allowed for MS and MS/MS, respectively. 1% FDR was used as a cutoff value for reporting identified peptides.

Data Submission

We have submitted the mass spectrometry data from this study in two public repositories. The raw data can be downloaded from Tranche data repository using the following hash code (F1iMznHQJ8+E+yfFk3nYyXTiQOX9IeTtBy/JTiqwpPNM-91Uf3h4onhabWYsMLsGaYzFLkNgYod9lZ5iARFH6Pyhm3FkAAAAAAAAXZQ==). The peptide identifications and associated MS/MS spectra are available from Human Proteinpedia[25] (HuPA_00672).

Bioinformatics Analysis

Human Protein Reference Database (HPRD)[26] was used for obtaining annotations pertaining to domains, motifs and post-translational modifications among the proteins identified. Gene ontology annotation was carried out by mapping the proteins identified to HPRD database which complies with GO terminologies for functional description of proteins.

RESULTS AND DISCUSSION

Urine pooled from 24 healthy individuals was used in this study. We employed SDS-PAGE-based separation of unfractionated as well as lectin-enriched urine samples as shown in Figure 1. In total, 54 in-gel bands were analyzed as two replicate runs by LC-MS/MS analysis using high resolution Fourier transform mass spectrometry. The acquired spectra were searched against Human RefSeq release 40 database using Sequest and Mascot search engines. The peptide identifications from Mascot and Sequest were combined into a single list each for both nonenriched and lectin-enriched data sets. A nonredundant list of peptide identifications was then obtained by merging the two lists from nonenriched and enriched data (Supplementary Table 2A, Supporting Information). If a given peptide was identified from both non enriched and enriched set, the one with the highest score was retained in the final compilation of urinary proteome. Supplementary Table 2B and 2C (Supporting Information) also provide a unique list of peptide identifications from enriched and

nonenriched data set. A complete list of proteins identified in this study is given in Supplementary Table 3, Supporting Information.

LC–MS/MS analysis of 30 in-gel bands from unfractionated urine without lectin-enrichment gave rise to the identification of 1452 proteins. The enrichment of glycoproteins in urine by a lectin-based affinity method using a mixture of three lectins, concanavalin A, jacalin and wheat germ agglutinin, was carried out to increase the depth of our analysis. Since different lectins have affinity toward different carbohydrate moieties, the use of multiple lectins in this step ensures a broader coverage of glycoproteins.[27] The enriched glycoproteins were subjected to SDS-PAGE. LC–MS/MS analysis of 24 in-gel digested protein bands from the lectin-enriched samples led to the identification of 617 proteins out of which 246 proteins were also identified in the unfractionated urine without lectin-enrichment. A list of all the proteins that were enriched by lectin affinity chromatography in this study is provided in Supplementary Table 4 (Supporting Information). In total, we identified 1823 proteins which include 721 single peptide hits. The single peptide hits were taken in to account only if it maps uniquely to the protein. Fifty percent of those hits were also supported by multiple peptide spectral matches (PSMs). Representative MS/MS spectra for a subset of proteins identified based on single peptide are shown in Supplementary figures (Supplementary Figure 1 and Supplementary Figure 2, Supporting Information).

The proteins that were identified in lectin-enriched fractions were mapped to HPRD, which contains manually curated literature-based evidence for post-translational modifications of human proteins.[28] Among the 617 proteins that were enriched, 265 proteins were known to be glycosylated based on data from HPRD. Though we did not enrich for acetylated peptides, we could find many peptides that were acetylated. In total, 131 peptides representing protein N-terminus were identified out of which 44 peptides were found to be acetylated.

Comparison with Other Studies on Urinary Proteomes

A number of studies have been carried out to characterize the urinary proteome from healthy individuals. Table 1 summarizes the studies carried out to date. We compared the list of proteins identified in our study with two other large-scale studies—one by Adachi et al. that reported 1543 proteins and another by Li et al. that reported 1310 proteins[16] (Figure 2). As seen in the figure, our study identified 744 proteins that were not reported by either of these two studies. Similarly, Li et al. identified 308 unique proteins and Adachi et al. identified 373 unique proteins. Proteins identified in common (658) to all the three studies are provided in Supplementary Table 5 (Supporting Information) as it represents the most commonly identifiable proteins in urine. Combining all the data from the studies published to date, there are at least 2500 proteins that have now been reported in the urine.

In addition to proteins being secreted in urine, exosomes are released into the urine by renal epithelial cells. Exosomes are known to be released by multiple cell types and have gained more attention after their discovery in many body fluids. Exosomes have been implicated in multiple biological processes including immune response, intracellular communication and transport.[29] Large scale analysis of exosomes in urine was carried out by Pisitkun et al. and Gonzales et al.[30, 31] By comparing our data with proteins identified in urinary exosomes, we

found that ~50% of the proteins identified in our study overlap with that identified in exosomes (Supplementary Table 6, Supporting Information).

Comparison of Urinary Proteome with Plasma Proteome

Lulu et al.[32] compiled the data from previously reported studies on urine and plasma and compared the proteins in kidney input (plasma) with those in the kidney output (urine). This study classified the proteins into three categories—proteins that were detected in urine, proteins that were detected in plasma and proteins that were detected in both plasma and urine. By comparing the list of proteins that was identified in this study with these three categories, we observed that 154 proteins which were previously thought to be restricted to the plasma proteome were detected in our analysis of the normal urinary proteome. Moreover, more than 90% of these proteins were identified for the first time in this study in urine. In summary, 23% (431/1823) of the proteins identified in urine were in common to the proteins that were reported in plasma.

Proteins Not Previously Reported in Urine

Of the 1823 proteins identified in our study, approximately 40% of the proteins were unique to our study. We think that this is likely owing to the fact that we employed an additional enrichment methodology. In fact, 25% of the novel proteins identified in this study have been identified from the lectin enriched fraction. In addition, we used an LTQ-Orbitrap Velos while many of the earlier studies were done on LTQ-Orbitrap or other instruments with a lower sensitivity. The significance of some of the identified proteins is discussed in detail below. A partial list of novel proteins identified in this study is given in Table 2. Representative MS/MS spectra of a subset of peptides identified from some of those proteins are shown in Figure 3.

Among the novel proteins detected in urine, we identified a group of voltage gated potassium ion channels which includes potassium voltage gated channel, subfamily E member 4 (*KCNE4)*, potassium channel tetramerising domain containing 12 (*KCTD12)* and potassium voltage gated channel, subfamily H member 5 (*KCNH5*). Voltage gated potassium channels are multifunctional and they play a crucial role in electrolyte transport, neuronal excitability and several other functions.[33] Germline variants in *KCNE4* have been shown to be associated with childhood acute lymphoblastic leukemia.[34] *KCNH5*, has been shown to hypermethylated in lung carcinoma as compared to normal lung tissue.[35] Ion channel proteins are an important class of molecules involved in multiple biological functions and their detection in urine might prove to be clinically significant. Shroom family member 2 (*SHROOM2*) was originally identified as human homologue of *APX* gene and is expressed in retina and other tissues including lung, kidney and pancreas. Based on its association with actin it has been characterized as a structural protein,[36] though the other functions of this protein are yet to be characterized. Shroom2 is known to cause amiloride sensitive sodium channel activity in *Xenopus laevis*.[37] It has also been reported as a strong candidate gene for ocular albinism.[36] A representative MS/MS spectrum of a peptide identified from Shroom2 is shown in Figure 3A.

Tumor suppressor candidate 1 (TUSC2) is another novel protein which was detected in urine. The gene encoding this protein is known to be deleted resulting in loss of heterozygosity observed in lung cancer cell lines.[38] A representative MS/MS spectrum of a peptide identified from TUSC2 is shown in Figure 3B.

Functional Classification of Identified Proteins

The proteins identified from urine were classified according to Gene Ontology (Figure 4). The classification based on cellular component (Figure 4A) revealed that the majority of proteins were known to be either extracellular (38%) or present on the plasma membrane (31%). We also identified a number of intracellular proteins that were known to be cytoplasmic (17%), lysosomal (6%) or nuclear (6%). Classification based on molecular function (Figure 4B) shows that the large majority of the proteins are known to be involved in cell–cell communication (27%), cell growth (15%) or metabolism or energy pathways (18%). Eighteen percent of the proteins were also known to be involved in transport (9%) and immune response (9%). Based on biological process (Figure. 4C), the proteins were classified into those involved in receptor activity (25%), cell adhesion activity (10%), growth factor activity (2%), catalytic activity (10%) and transporter activity (9%) and as part of extracellular matrix structural constituent (7%).

Potential Biomarkers Identified in Human Urine

The proteins that we have detected in normal urine could be altered in their expression in various diseases in which case the differential expression of such proteins could be assessed in urine. This could potentially lead to development of sensitive assays as markers for such diseases. An overview of some of the proteins identified in our study that have previously been reported to be altered in abundance in certain disease conditions is provided below.

Fatty acid binding proteins (FABPs) are a group of small molecular weight proteins that are involved in lipid metabolism and transport.[39] Nearly 10 members of this family of proteins have been identified thus far.[39] Among them, FABP1, FABP2, FABP3 and FABP5 were detected in human urine under normal conditions in this study. Few of the proteins that were mentioned above have been described in the context of disease conditions. FABP1 has been described as a biomarker for early detection of acute kidney injury and chronic kidney disease.[40] FABP3 was discovered as a diagnostic marker in cerebrospinal fluid of patients with stroke as well as in the serum of those patients.[41]

We were able to detect several proteins implicated in various cancers in human urine. For instance, tubulin (*TUBB*), beta actin (*ACTB*), and vimentin (*VMN*) that have been shown to be overexpressed in hepatacellular carcinoma[42] were detected in urine. Several other proteins—heat shock 70 kDa protein 8 (*HSPA8*), annexin A4 (*ANXA4*), tubulin beta (*TUBB2C*), 14-3-3 beta (*YWHAB*)—that have been reported to be overexpressed in renal cell carcinoma[43] were detected in urine. We were able to identify proteins like *FABP5* and Ezrin (*EZR*), which are known to be overexpressed in lymph node metastases of prostate cancer.[44] We could also detect tumor suppressor candidate 2 (*TUSC2*) (Figure 3B) in urine, which has been found to be downregulated in lung cancer.[45]

Kinases are key molecules involved in signaling pathways regulating cellular proliferation, metabolism, differentiation and survival. Identification of these essential proteins in body fluids would lead to novel methods of clinical diagnosis. Some of the kinases that were identified in urine are discussed below. Ephrin receptors are the largest known subfamily of receptor tyrosine kinases. In mammals, Ephrin receptor family comprises of 14 members.[46] Ephrin receptors have been shown to act as both tumor promoters and tumor suppressors in different cancers.[46] We identified a number of ephrin receptor family members – Ephrin receptors A1, A2, A7, A10, B2, B3, B4 and B6 (*EPHA1, EPHA2, EPHA7, EPHA10, EPHB2, EPHB3, EPHB4*, and *EPHB6*) in urine. Ephrin receptor A2 (*EPHA2*) and *EPHA7* identified in our study in urine have been shown to be abundantly expressed in pancreatic ductal adenocarcinoma tissues.[47] Other kinases that were overexpressed in cancers,[48, 49, 50] and detected in urine include- AXL receptor tyrosine kinase (*AXL*), PDGF receptor beta (*PDGFRB*) and fibroblast growth factor receptor 1 (*FGFR1*).

Glycoprotein Markers Identified in Human Urine

A large majority of the secreted and cell surface proteins that are shed into the bloodstream are known to be glycosylated.[51] A number of these proteins were detected in the lectin-enriched fraction from urine. Some of the serum glycoproteins[51] that were detected in urine include alkaline phosphatase (*ALPP*), uromodulin (*UMOD*), carcinoembryonic antigen 5 (*CEACAM5*) kallikreins 3, 5, 6, 7, 8, 10, and 11 (*KLK3, KLK5, KLK6, KLK8, KLK10* and *KLK11* (Figure 3C)) and vitamin D binding protein (*GC*). Apolipoprotein A (*APOA*), apolipoprotein A2 (*APOA2*), and haptoglobin (*HP*), which have been reported to be elevated in the serum of hepatocellular carcinoma patients,[42] tissue inhibitor of matrix metalloproteinase 1 (*TIMP1*), which has been reported to be elevated in the serum of colon cancer patients,[52] mac-2 binding protein (*LGALS3BP*), which has been reported to be elevated in the secretome of esophageal squamous cell carcinoma cells,[53] were also detected in the lectin-enriched fraction.

Interestingly, we could identify proteins that are associated with neurological disorders in urine. One such protein is folate receptor alpha (*FOLR1*), which has recently been described in the context of meningomyelocele, a developmental birth defect caused by the incomplete closure of the neural tube.[54] We were able to detect two members of the family in urine—folate receptor 1 (*FOLR1*) and folate receptor 2 (*FOLR2*) (Figure 3D). Another protein sortilin (*SORT1*) which was detected in urine has been described in the context of Alzheimer's disease.[55] It has also been described in association with coronary artery disease[56] and myocardial infarction.[57]

CONCLUSIONS

We report here the largest catalog of human urinary proteome to date based on high confident peptide identification. This was achieved by the use of high-accuracy and high-resolution mass spectrometer. In spite of urine being available by noninvasive methods, its diagnostic potential has not been exploited except in few cases like pregnancy test and other kidney-related diseases. Findings from this study could provide a basis for biomarker assay developments in various diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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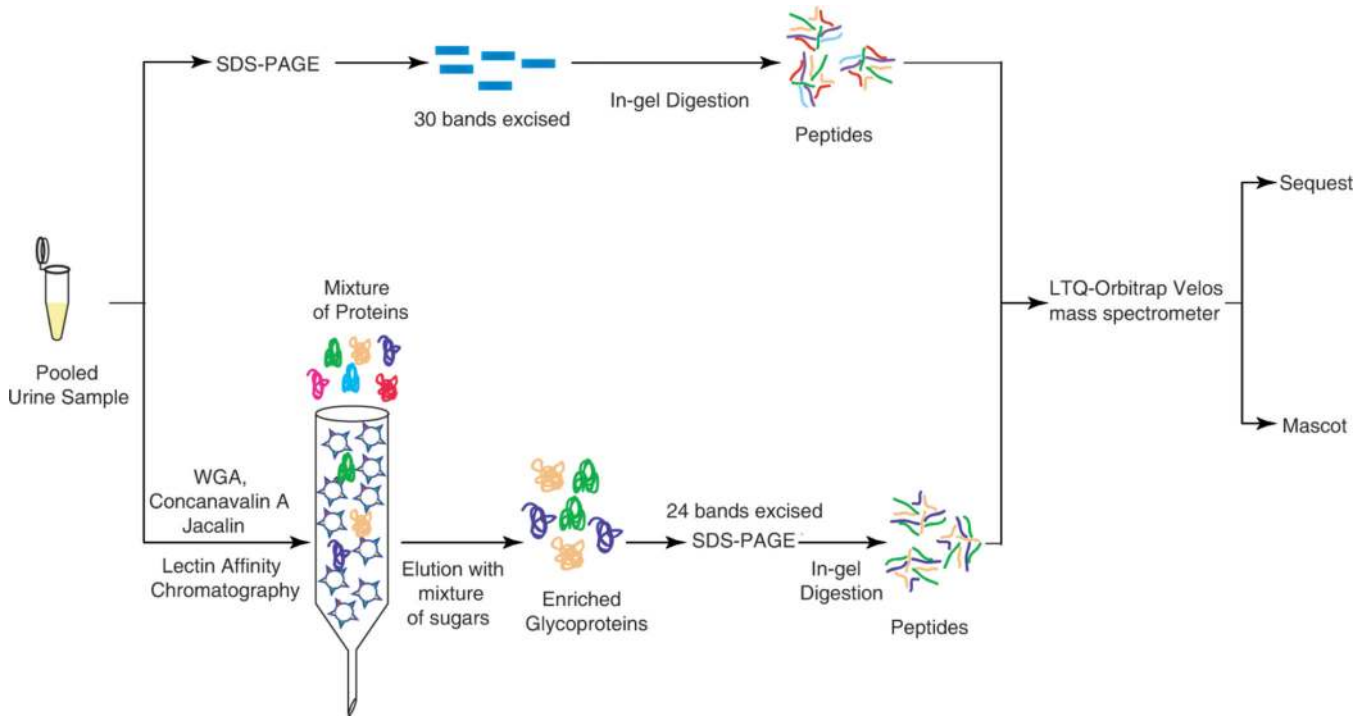

Figure 1.

Workflow employed to characterize the urinary proteome. Pooled urine from healthy individuals was processed by two different approaches. A fraction of pooled urine was resolved by SDS-PAGE without any enrichment. Gel bands were excised followed by in-gel digestion. Another fraction was subjected to lectin affinity chromatography using three lectins (Concanavalin A, wheat germ agglutinin and jacalin) followed by SDS-PAGE and in-gel digestion. In-gel digested gel bands from two different approaches were further analyzed by liquid chromatography tandem mass spectrometry (LC–MS/MS) on an LTQ-Orbitrap Velos mass spectrometer. Data derived from mass spectrometric analysis were then searched using Mascot and Sequest search engines.

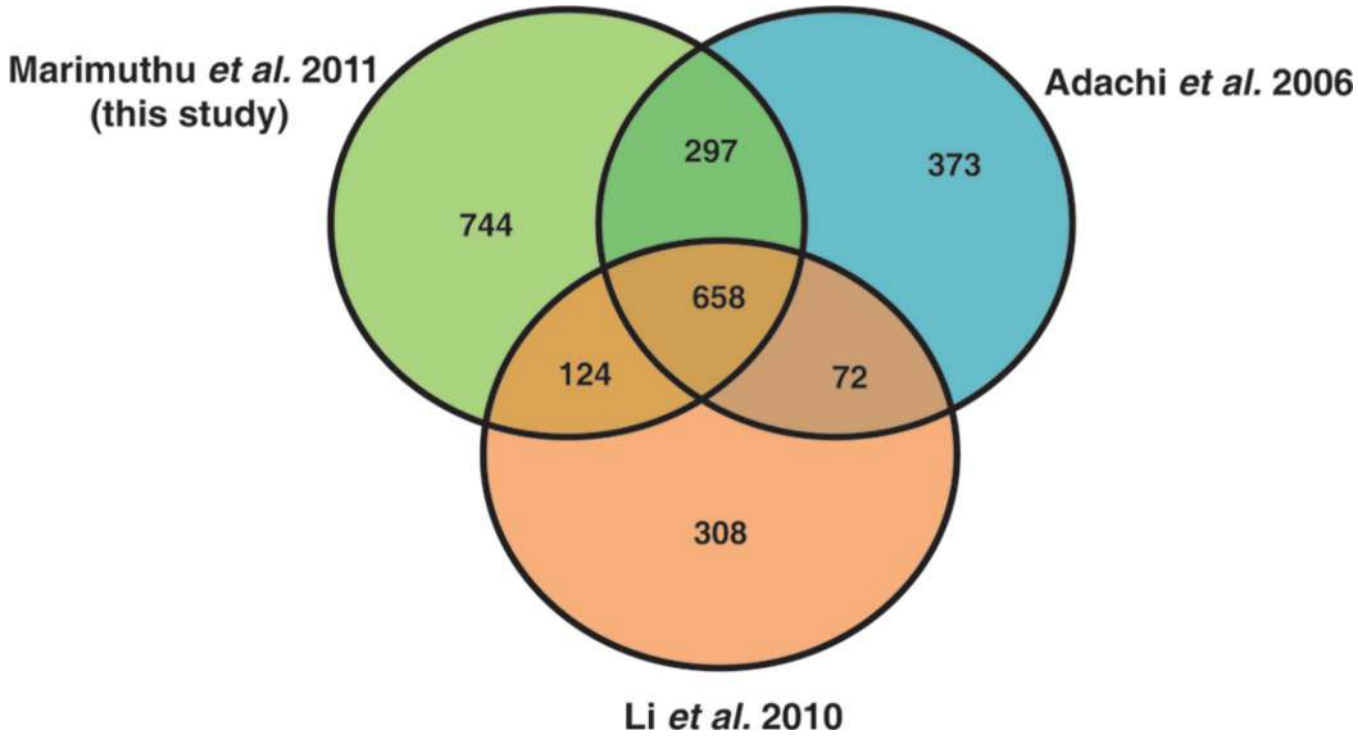

Figure 2.

Venn diagram comparing large scale studies on urinary proteome. Protein identifications from the current study were compared to two other studies (Adachi et al., 2006 and Li et al., 2010) that were carried out using high-resolution mass spectrometers. A total of 744 proteins were unique to this study, whereas 373 proteins were unique to Adachi et al. and 308 were unique to Li et al.

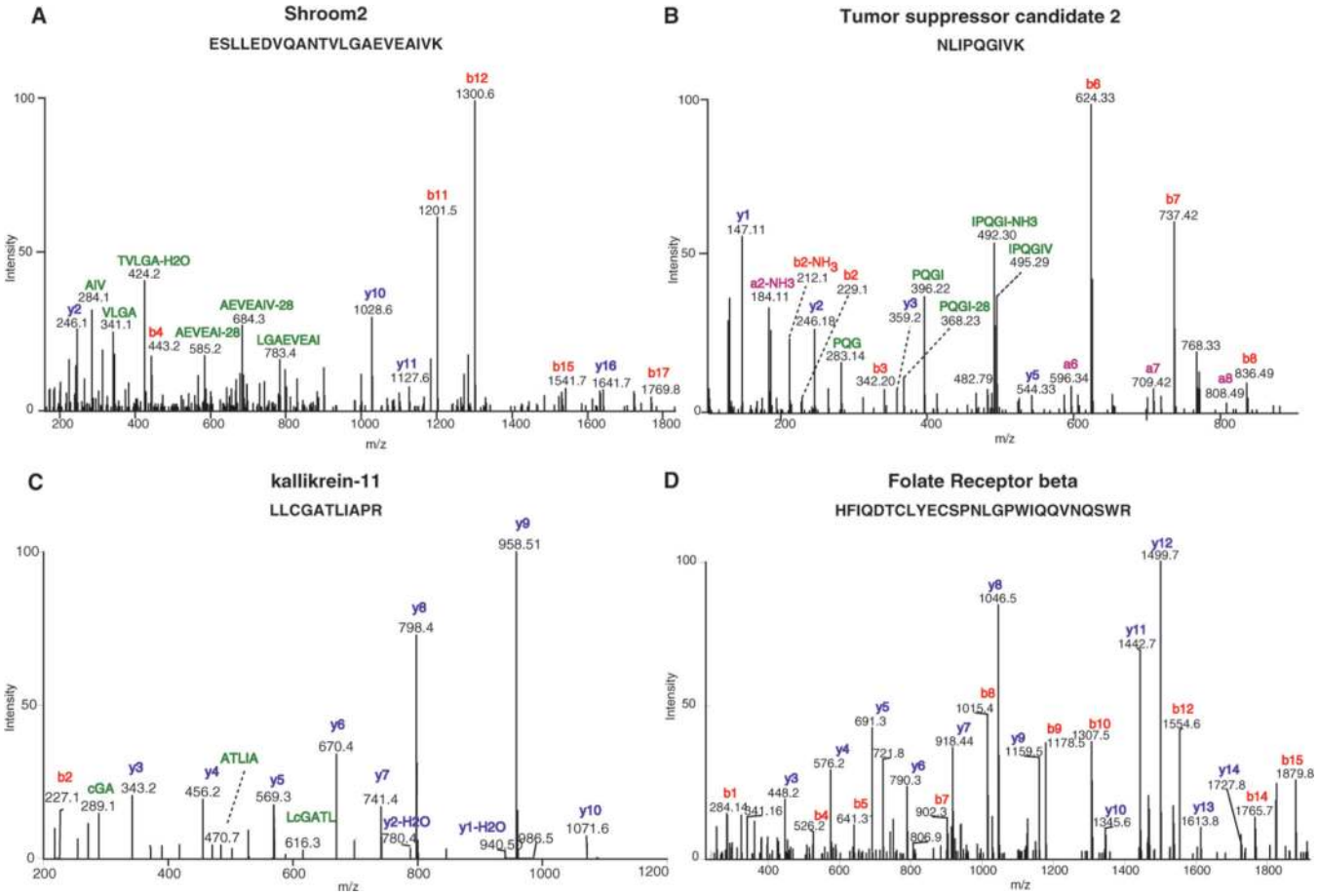

Figure 3.

Representative MS/MS spectra of peptides from selected proteins that were identified based on single peptide evidence. (A) Shroom2 (*SHROOM2*). (B) Tumor suppressor candidate 2 (*TUSC2*). (C) Kallikrein-11. (D) Folate receptor beta (*FOLR2*).

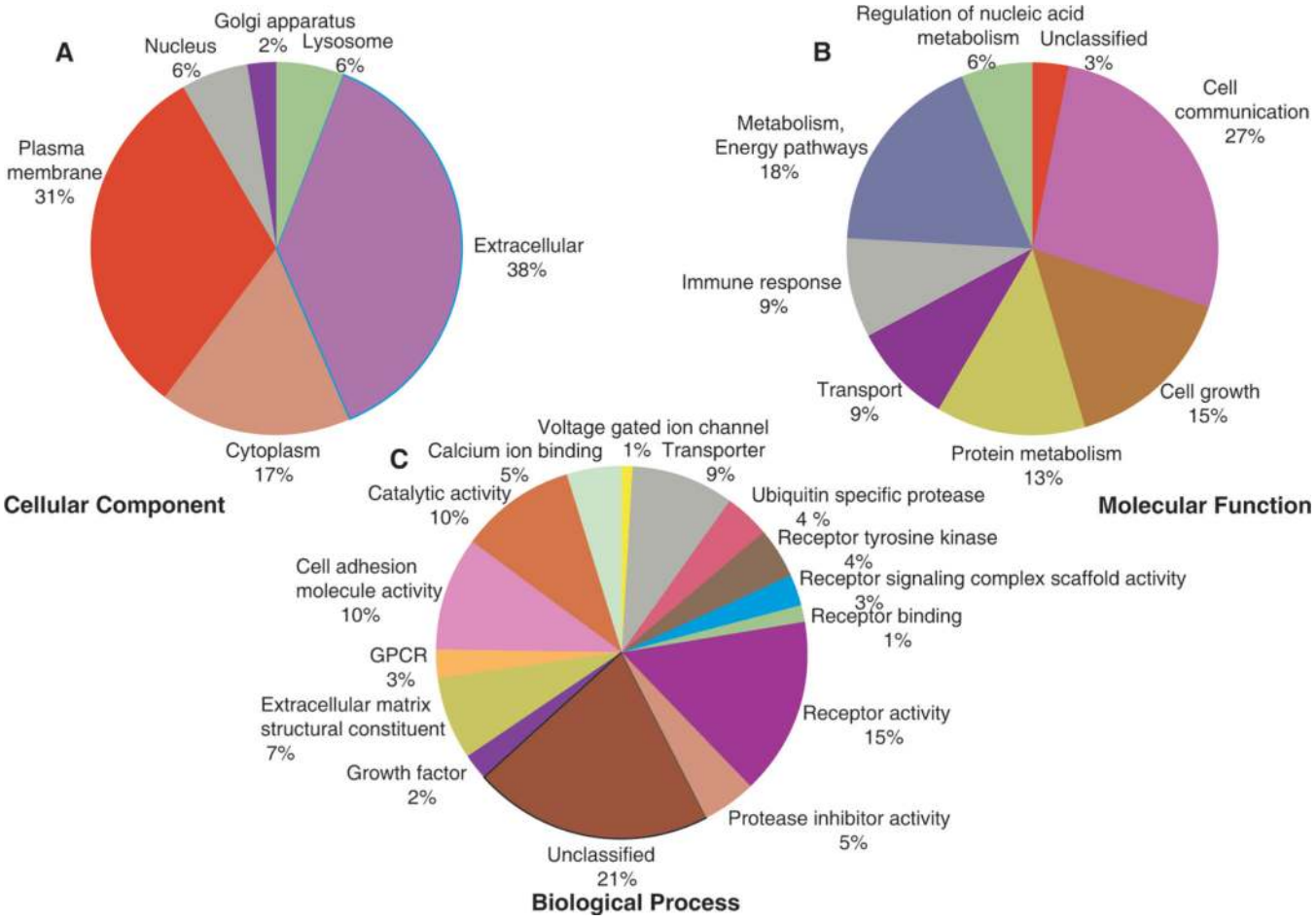

Figure 4.

Gene Ontology based classification of proteins. (A) Cellular component, (B) molecular function and (C) biological process based classification of identified proteins in this study.

Table 1

Proteomic Studies Carried out on Healthy Human Urine[a]

S.NO	total/sub proteome	depletion/enrichment	number of identifications	instrument used	citation
1	Total	Albumin depleted	1543	LTQ-Orbitrap	Adachi et al. (2006)
2	Total		1310	LTQ-Orbitrap	Li et al. (2010)
3	Exosomes		1132	LTQ linear ion trap	Gonzales et al. (2009)
4	Total		600	LTQ	Lee et al. (2008)
5	Total	Ligand library beads enrichment	383	SELDI-TOF and FT-ICR	Castagna et al. (2005)
6	Total		339	MALDI-TOF	Khan et al. (2006)
7	Total	Lectin affinity enrichment	334	LCQ-DECA XP Plus	Wang et al. (2006)
8	Exosomes		295	LCQ-DECA XP	Pisitkun et al. (2004)
9	Total		245	LCQ-DECA XP	Kim et al. (2009)
10	Total		226	LCQ-DECA XP Plus	Sun et al. (2005)
11	Total	Immunoaffinity subtraction chromatography	150	MALDI-TOF and LCQ-DECA	Pieper et al. (2004)
12	Total	Combination of LDS-PAGE and 2-DE	141	MALDI-TOF/TOF	Zerefos et al. (2006)
13	Total		124	Q-TOF	Spahr et al. (2001)
14	Total		113	MALDI-TOF	Oh et al. (2004)
15	Total	None	91	LCQ-DECA	Pang et al. (2002)
16	Total	-	48	MALDI-TOF/TOF	Smith et al. (2005)
17	Total	None	47	MALDI-TOF	Thongboonkerd et al. (2002)

[a]Summary of the mass spectrometry based studies carried out until date to identify urinary proteome of healthy individuals is listed. Table includes details about depletion or enrichment strategy that was employed, number of protein identifications and the type of mass spectrometer used in the listed studies.

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Table 2

Partial List of Proteins Not Previously Reported in Urine[a]

gene symbol	name	subcellular localization	molecule class	molecular function
BAI2	Brain specific angiogenesis inhibitor 2	Plasma membrane	Integral membrane protein	Receptor activity
FOLR2	Folate Receptor 2	Plasma membrane	Cell surface receptor	Receptor activity
GPC6	Glypican 6	Plasma membrane	Growth factor	Growth factor activity
LYST	Lysosomal trafficking regulator	Lysosome	Adapter molecule	Receptor signaling complex scaffold activity
ST6GAL1	Sialyltransferase 1	Golgi apparatus	Enzyme: Sialyltransferase	Sialyltransferase activity
LCN6	Lipocalin 6	Extracellular	Unclassified	Molecular function unknown
EGFL6	Epidermal growth factor-like 6	Extracellular	Secreted polypeptide	Molecular function unknown
IGFBP4	IGF binding protein 4	Extracellular	Adhesion molecule	Cell adhesion molecule activity
MUCL1	Small breast epithelial mucin	Extracellular	Secreted polypeptide	Molecular function unknown
NCAN	Neurocan	Extracellular	Extracellular matrix protein	Extracellular matrix structural constituent
SPP2	Secreted phosphoprotein 2, 24-KD	Extracellular	Protease inhibitor	Protease inhibitor activity
TYRP1	Tyrosinase related protein 1	Endosome	Oxidoreductase	Oxidoreductase activity
SHISA5	Scotin	Endoplasmic reticulum	Integral membrane protein	Signal transducer activity
CHMP4A	Charged multivesicular body protein 4a	Cytoplasmic vesicle	Unclassified	Molecular function unknown
CAMKK1	CAMKK alpha protein	Cytoplasm	Serine/threonine kinase	Protein serine/threonine kinase activity
TUSC2	Lung cancer candidate FUS1 protein	Cytoplasm	Unclassified	Molecular function unknown
CIT	Citron	Cytoplasm	Serine/threonine kinase	Protein serine/threonine kinase activity
FICD	Huntingtin interacting protein E		Unclassified	Molecular function unknown
MEAF6	Sarcoma antigen NY-SAR-91		Unclassified	Molecular function unknown
TCHH	Trichohyalin		Calcium binding protein	Calcium ion binding

[a]A list of novel proteins with their subcellular localization, molecular class and molecular function are provided.