Chapter 18
Comparing Plasma and Urinary Proteomes to Understand Kidney Function

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Abstract Kidney function is rarely studied in the context of blood kidney and urine as a system. Kidney can be considered as a black box, while plasma and urine proteomes closely represent the protein compositions of the input and output of the kidney. This idea provides a new approach for studying organ functions with a proteomic methodology. Because of its distinctive input (plasma) and output (urine), it is reasonable to predict that the kidney will be the first organ whose functions are further elucidated by proteomic methods in the near future.

Keywords Kidney function · Black box

18.1 Introduction

A large volume of plasma (350–400 mL/100 g of tissue per min) is filtered by the kidney to generate about 150–180 L/per day ultrafiltrate, and then, most components in the ultrafiltrate are selectively reabsorbed until less than 1 % of the ultrafiltrating volume is excreted as urine [13]. In this physiological process, the plasma proteins are filtered by the kidney. After handling by the kidney including reabsorption and secretion, the urine proteins were produced and excreted. In short, the plasma proteins enter the kidney, then the urine proteins out. The kidney can be regarded as a black box with distinct input and output proteomes. Therefore, the kidney's protein handling function can be studied by comparing plasma and urine proteomes.

The plasma proteome could be regarded as the input proteome. However, the urine proteome could not be simply regarded as the kidney output proteome due to its complicated protein sources. In addition to the kidney, urine proteins may also

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Fig. 18.1 Kidney function analysis by black box method

be derived from the urine tract and glands downstream of the kidney [9, 11]. Proteins from latter sources might complicate the kidney output. For black box analysis, all input should go into the black box, and all output should come directly out of the black box. For analysis of the kidney's protein handling function, available proteins secreted into the urine downstream of kidney were subtracted from the plasma and urine proteomes to form the effective input and output proteomes, respectively (Fig. 18.1) [3].

By comparing these modified kidney input and output proteomes, this work first aims to find which proteins are blocked or permitted to pass through and which proteins are secreted or shed from the kidney. Because these different protein handling pathways in the kidney are closely related to the form and size of individual proteins, the experimental molecular weights (MWs) of proteins are therefore important for understanding the mechanisms of protein handling by the kidney. Then, the experimental MWs of the proteins in plasma and urine are compared. For different proteins, the associations between the quantitative changes from plasma proteome to urine proteome and their MWs were also investigated.

18.2 Kidney Input Proteome and Output Proteome

The human plasma proteins were collected from the plasma proteome project (PPP) initiated by HUPO, who published a high confidence (FDR 1 %) list of 1,929 identified proteins (http://www.mcponline.org/content/suppl/2011/06/01/mcp.M110.006353.DC1/mcp.M110.006353-4.xls) including Phase I and II data, in June 2011 [Farrah et al. (http://www.mcponline.org/content/early/2011/06/01/mcp.M110.006353.long)]. The human urine proteins were collected from three large-scale datasets of the previous studies [1, 6, 7] and one large-scale dataset identified in our institution (data not published). There are only a limited number of proteins with experimental MWs information published so far in plasma and urine. In this study, the experimental MWs of the proteins were collected from the previous study [4, 8, 10].

The prostate is a secretory gland downstream of the kidney that might secrete prostatic fluid into urine, which would complicate the kidney output proteome. Human prostatic secretion proteins identified by proteomic methods were hence removed from the urine proteome and plasma proteome. A total of 114 human prostatic secretion proteins were acquired from Lin et al. [5]. Other proteins that are possibly incorporated in urine downstream of kidney were temporarily ignored due to limited knowledge at this time.

For easier comparison, the protein identifiers in different datasets were standardized. The UniProt Reference Clusters (UniRef) provide clustered sets of sequences from the UniProt Knowledgebase (http://www.uniprot.org/help/uniprotkb) [including isoforms (http://www.uniprot.org/help/canonical_and_isoforms)] while hiding redundant sequences. UniRef90 was selected as the reference database. All the protein identifiers of different datasets were mapped to UniRef90 cluster using ID mapping tool from UniProt Web site. After protein ID standardization and removal of prostatic secretion proteins, kidney input and output proteome were generated. This resulted in 1,778 non-redundant UniRef90 sequences in the plasma as the kidney input proteome and 6,093 non-redundant UniRef90 sequences in the urine as the kidney output proteome.

18.3 Kidney Function Described in Proteomic Language

The plasma-only subproteome, the plasma-and-urine subproteome, and the urine-only subproteome were generated after comparing the kidney input and output proteomes with the kidney considered as a black box. The function of the kidney can be described in itemized proteomic language as whether a particular protein is blocked, permitted to pass, or secreted/shed from the kidney. These three groups of proteins correspond to the three subproteomes.

18.3.1 Plasma-Only Subproteome

There were 351 non-redundant protein sequences in the plasma-only subproteome. Currently, there was no evidence that they were present in urine based on available proteomic data. Due to the profiling depth of the urine proteome is significantly exceeded that of the plasma proteome, this group of proteins is more reliable. These proteins are supposed to be difficult to pass through the kidney black box. There were only 4 proteins with experimental MWs, which were relatively small proteins. They were P11226 (26.3 kDa), P02745 (26.3 kDa), P20742 (51.6 kDa), and Q14289 (65.1 kDa). The theoretical MWs were in a range from 3 kDa to more than 400 kDa, while the theoretical PIs were in a range from 4 to 11.6. According to estimated concentration of the proteins in plasma provided by plasma proteome dataset, there were many moderate- or even high-abundant proteins that were present only in plasma but not in urine. Particularly, there were some high-abundant Ig proteins present only in plasma. This group included proteins that could not be filtered at the glomerular capillaries or filtered but reabsorbed completely back into the blood from the tubules.

18.3.2 Plasma-and-Urine Subproteome

There were 1,424 non-redundant protein sequences that existed in both plasma and urine. They may pass the kidney black box in various forms. There were only 15 proteins with experimental MW information available from both plasma and urine, which range from 11 to 133 kDa in plasma and 11 to 77 kDa in urine. Comparing their MWs in plasma and in urine, six proteins were within 20 % variation, suggesting that they may pass through the kidney in an intact form; four had MWs 20 % higher in plasma than in urine, and five had MWs 20 % lower in plasma than in urine. These differences reflect functions of the kidney. In addition to one protein whose theoretical MW was 3,816 kDa, the theoretical MWs of the proteins were in a range from 3 kDa to more than 600 kDa, while the theoretical PIs were in a range from 3.6 to 12.

For the kidney considered as a black box, the quantitative changes of different proteins from plasma proteome to urine proteome reflect the kidney protein handling function. Therefore, the ranking order of the plasma and urine proteins sorted by their concentrations in plasma was compared with that in urine. The estimated concentrations of the proteins in plasma were provided by plasma proteome dataset, which were used to generate their ranking order in plasma. Many urine proteins were collected from a large-scale dataset identified in our institution using MASCOT search engine (data not published). The MASCOT search engine has been incorporated the exponentially modified protein abundance index (emPAI), which offers approximate, label-free, relative quantitation of the proteins in a mixture based on protein coverage by peptide matches [2]. Each identified urine protein had an emPAI value, which can be used to approximately estimate the absolute protein contents in urine. The emPAI values of these plasma and urine proteins were extracted from the large-scale dataset. These proteins were sorted from most to least abundant. Proteins not identified in the large-scale dataset were at the end. The ranking order of these proteins approximately represents their abundance in human urine. After comparing their ranking order in plasma with that in urine, we found that the two ranking orders did not correspond well with each other. Many proteins have a significantly changed ranking order when they passed through the kidney. This suggested that the kidney performed different handling functions for the different proteins.

It is believed that proteins with a MW of <15 kDa are freely filtered in the glomeruli; proteins up to 45 kDa are quite rapidly filtered and proteins between 45 and 60 kDa only restrictedly. Plasma proteins larger than 60 kDa are not filtered through the kidney [12]. We found that some proteins with experimental MW <45 kDa exist in the plasma but have not been identified in the urine proteomic data until now. Particularly, some moderate-abundant and high-abundant proteins even they are low molecular weight were present only in plasma but not in urine. There were some possible mechanisms. For example, they might bind to larger carrier proteins or there might be some unknown mechanisms for them to be retained for an extended period in the plasma. We have also found that some

proteins with experimental MW >60 kDa had been identified both in plasma and in urine such as P35858 (experimental molecular weight 80 kDa in plasma and 77 kDa in urine) and P05155 (experimental molecular weight 91 kDa in plasma and 75 kDa in urine). These proteins might be secreted, but the passing through the glomeruli could not be ruled out. This is worth of further study. When the proteins passed through the kidney, the different quantitative changes of the proteins from plasma proteome to urine proteome reflect the different handling function of that particular protein by kidney.

18.3.3 Urine-Only Subproteome

Four thousand one hundred and eighty-one proteins were identified only in urine, but not in plasma, by proteomic methods. Proteins secreted or shed from the kidney are thought to be included in this group. Since the profiling depth of the urine proteome is higher than that of the plasma proteome, there were potentially a lot of false-positive proteins. In other words, some of the 4,181 proteins might exist in plasma but were missed, so they may have been determined to belong to plasma-and-urine subproteome instead of urine-only one.

18.4 Discussion and Perspective

Since mass spectrometry-based proteomics was founded, body fluid proteomes, such as plasma, urine, tear, and cerebrospinal fluid, have been profiled by many groups. All of the body fluids interact with each other and the organs and finally collectively contribute to form a dynamic system in the body. For instance, plasma may influence most other body fluids, such as urine, cerebrospinal fluid, and tears. It is important to analyze the proteomes of various body fluids in the context of plasma. Theoretically, the difference between any two body fluid proteomes can reflect the function of that part of the body between them. Particularly for the kidney, there were two obviously different input and output proteomes. The kidney protein handling function can be studied by comparing plasma and urine proteome.

Though proteomics has been improving rapidly, it is probably still far from being capable of exhaustively identifying all proteins in plasma and urine. Here, the comparison method described in this paper provides an illustration of a new approach for studying organ functions with a proteomic methodology. In the future, plasma and urine samples from one individual at the same time point can be characterized for the study of an individual's kidney function. Sex-specific proteins, presumably coming from sex-specific glands, can be identified if the male and female proteomes are profiled separately and they should be removed from the kidney output proteome for the black box study of kidney function. It would be better to compare the two proteomes using unbiased quantitative proteomic

techniques. With further development of proteomic technologies, i.e., quantitative-MS-based proteomics, top-down strategy proteomics, and antibody arrays, and improvement in the data quality, such comparisons will presumably result in more meaningful and valid conclusions. More detailed descriptions of kidney functions can be obtained by comparing two or more proteomes with more exhaustive and reliable protein information, such as complete MWs, pIs, posttranslational modifications, and quantitation. Because of its distinctive input (plasma) and output (urine), it is reasonable to predict that the kidney will be the first organ whose functions are further elucidated by proteomic methods in the near future. It can also be anticipated that there will be more applications for proteomics in organ function research.

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