Chapter 9
Variations of Human Urinary Proteome

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Abstract The study on dynamic analysis of human urinary proteome is the foundation that we discriminate certain various urinary proteins as potential biomarker derived from the disease itself or normal physiological change. In our results, based on RPLC-MS/MS and spectral count to study pooled and individual urine samples and other researchers' studies, it can be known that the content of many urinary proteins maintain relatively stable. We have reason to believe that the relatively stable urinary protein is a very valuable resource as biomarkers. Many similar proteins such as prostaglandin-H2 D-isomerase and apolipoprotein D proteins have been proved our hypothesis. The following field, the number, preservation and treatment methods of urine sample, the standardization of analysis method and data processing, and suitable quantitative method, is ought to the focus of future study.

Keywords Variation · Human urine proteome

The blood and urine composition remains on the correlation because of the renal filtration, and human urinary proteome analysis is a convenient and efficient approach for understanding disease processes affecting the kidney and urogenital tract.

During the biomarker discovery, dynamic variations of the urinary proteomic compared with cerebrospinal fluid and tissue samples are a more important issue. The changes in urine on a certain extent reflect the physiological status in the body. The urinary proteins list can be diversified by diet, medicines, daily activities, menstrual cycle, and other physiological conditions as well as environmental factors such as temperature and humidity. The range of urinary proteomic determines whether it has enough stability as clinical biomarker.

There are hundreds of proteins which were found in the human urine proteome at beginning. Our understanding of normal urinary proteome has been further comprehensive with the technique diversification of proteomics research; especially,

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high-resolution mass spectrometer was employed. A total of 1,543 proteins in urine were identified from ten healthy donors by a linear ion trap–Fourier transform (LTQ-FT) and a linear ion trap–orbitrap (LTQ–orbitrap) mass spectrometer [1]. A total of 2,362 proteins were obtained in normal urine, by combination of various pre-separating means, such as ultracentrifugation, gel separation, ion exchange chromatography, and reverse-phase chromatography [6]. A considerable number of proteins identified especially trace proteins were analyzed by independent research. The difference comes possibly from the various analysis strategies, different samples, and nonstandard data processing, but it verified objectively the huge variability of urinary proteome. Therefore, it is important and necessary for the clinical diagnosis to standardize the sample preparation, mass spectrometry analysis, and data processing [2]. We recommend that researchers pay more attention to variability of the urine proteome in current and future study, especially quantitative analysis.

"**Population proteomics**" **can be used to study urinary variability**. It focuses urinary proteome on the population level and gives us the better understand the diversity of proteins in human urine [9, 10]. Population proteomics concerns the change of different protein individual in the population on the specific structure and the number of the protein. In the clinical proteomics study, the introduction of the population proteomics concept can be understood as the abundance change on different conditions or time points for specified proteins. For the existed clinical diagnosis biomarkers, its abundant change is the result of the normal physiological state changes, but pathological condition is not real cause! Due to the huge variation and individual feature of normal urine, the stable urinary proteins those have significantly qualitative and quantitative difference between pathological and physiological condition may have greater chances to serve as potential urinary biomarkers. For example, kidney damage prostaglandin-H2 D-isomerase was considered as relation of hypertension and diabetes induced kidney injury [3, 4]. Pancreatic ribonuclease and alpha-1 acid glycoprotein 1 are seen as potential diabetic nephropathy biomarkers [5]. Apolipoprotein D that involved in fat metabolism in the body was associated with a various tumors, and expression level was significantly reduced in hepatoma cells [13], and the abundance of Apolipoprotein D proteins also exhibits fluctuation in different phase in prostate cancer [11]. Obviously, the prostate-specific antigen as prostate cancer biomarker can be detected in almost all the men urine. Thus, the expression profile of normal urinary proteome, especially the relatively stable members, was ought to analyze quantitatively in the first place. It is the foundation that we discriminate certain various urinary proteins as potential biomarker were derived from the disease itself or normal physiological change. Therefore, we believe that the relatively stable urinary protein is a very valuable resource as biomarkers.

In our previous study, RPLC-MS/MS and spectral count as the semi-quantitative analysis were used to study human urinary proteome variation by pooled and individual urine samples. Five types of pooled urinary samples (first morning void, second morning void, excessive water drinking void, random void, and 24-h void) collected in 1 day from six volunteers (three males and three

females) were used to analyze the urinary proteome overall intraday variations. Six pair first morning urine collected on day 0 and day 7 from above six volunteers were utilized to study inter-day, inter-individual, and inter-gender variations. A total of 31 common proteins were detected in all urinary samples, and their CV about spectra count changes from 35.3 to 97.3 %, the median CV of 59.8 %. Spectra count between the minimum and maximum number was six less than 5 times, ten 5–10 times, and fifteen more than 10 times. The intraday, inter-day, inter-individual, and inter-gender variation results showed 50 % of proteins were found in various samples and more than 40 % of these proteins whose spectral count variation was less than two times. Based on the proteomics data of urine from six volunteers, the intraday variation is less than the inter-individual variation. Inter-gender stability is the worst [12]. These results suggest that the content of many urinary proteins maintain relatively stable.

The 20 urine samples from volunteers (10 males and 10 females) were collected, respectively, by Molina et al. [7]. The occurrence of the 910 identified spots was analyzed throughout the gels and represented in a virtual 2D gel. Sixteen percent of the spots were found to occur in all samples and 23 % occurred in at least 90 % of urines. About 13 % of the protein spots were present only in 10 % or less of the samples, thus representing the most variable part of the normal urinary proteome. The quantitative analysis data of stable proteins change in the large range. Furthermore, the content of "public" urinary proteins was more than "private" proteins.

In the similar studies, Adachi showed by LC-MS that the urinary proteome of a single person comprises a major set (61.9 %) of proteins that is also found in a pool of 9 urines [1]. By LC-MS/MS label-free quantification analysis on an LTQ–orbitrap without prefractionation, Nagaraj characterized the urinary proteome of seven normal human donors over three consecutive days [8]. A total of 600 proteins were identified, and 500 proteins were readily detectable in all studied individuals. Inter-day variability was markedly higher with a CV of 0.48, and the overall variation of the urinary proteome between individuals was 0.66. These results confirm that small amounts of specific proteins coexist with the large number of common proteins in normal human urine. From the methodological point of view, the 24 % common proteins was detected by 2D gel [7], and more than 60 % of the common protein was identified by LC-MS methods [1, 8].

In the case of few studies on dynamic analysis of human urinary proteome, there is a large difference and it is difficult to compare with each other. The complexity and variability of urinary proteome is also a very important reason in addition to the technical limitations of existing proteomic methods. The number, preservation and treatment methods of urine sample, the standardization of analysis method and data processing, and suitable quantitative method especially all seriously affect the quality of the final results. Meanwhile, it is the big bottleneck for high-throughput and quantitative analysis of a large number of clinical urine samples in a short time. It can be predicted that the results from urine samples of different race, gender, age, geography, lifestyle, and collection time will be more theoretical and practical value.

References

1. Adachi J, Kumar C, Zhang Y, Olsen JV, Mann M (2006) The human urinary proteome contains more than 1500 proteins, including a large proportion of membrane proteins. Genome Biol 7:R80
2. Fliser D, Novak J, Thongboonkerd V, Argiles A, Jankowski V, Girolami MA, Jankowski J, Mischak H (2007) Advances in urinary proteome analysis and biomarker discovery. J Am Soc Nephrol (JASN) 18:1057–1071
3. Hirawa N, Uehara Y, Ikeda T, Gomi T, Hamano K, Totsuka Y, Yamakado M, Takagi M, Eguchi N, Oda H, Seiki K, Nakajima H, Urade Y (2001) Urinary prostaglandin D synthase (beta-trace) excretion increases in the early stage of diabetes mellitus. Nephron 87:321–327
4. Hirawa N, Uehara Y, Yamakado M, Toya Y, Gomi T, Ikeda T, Eguchi Y, Takagi M, Oda H, Seiki K, Urade Y, Umemura S (2002) Lipocalin-type prostaglandin d synthase in essential hypertension. Hypertension 39:449–454
5. Jain S, Rajput A, Kumar Y, Uppuluri N, Arvind AS, Tatu U (2005) Proteomic analysis of urinary protein markers for accurate prediction of diabetic kidney disorder. J Assoc Phys India 53:513–520
6. Kentsis A, Monigatti F, Dorff K, Campagne F, Bachur R, Steen H (2009) Urine proteomics for profiling of human disease using high accuracy mass spectrometry. Proteomics Clin Appl 3:1052–1061
7. Molina L, Salvetat N, Ameur RB, Peres S, Sommerer N, Jarraya F, Ayadi H, Molina F, Granier C (2011) Analysis of the variability of human normal urine by 2D-GE reveals a "public" and a "private" proteome. J Proteomics 75:70–80
8. Nagaraj N, Mann M (2011) Quantitative analysis of the intra- and inter-individual variability of the normal urinary proteome. J Proteome Res 10:637–645
9. Nedelkov D (2005) Population proteomics: addressing protein diversity in humans. Expert Rev Proteomics 2:315–324
10. Nedelkov D, Kiernan UA, Niederkofler EE, Tubbs KA, Nelson RW (2006) Population proteomics: the concept, attributes, and potential for cancer biomarker research. Mol Cell Proteomics (MCP) 5:1811–1818
11. Rodriguez JC, Diaz M, Gonzalez LO, Sanchez J, Sanchez MT, Merino AM, Vizoso F (2000) Apolipoprotein D expression in benign and malignant prostate tissues. Int J Surg Invest 2:319–326
12. Sun W, Chen Y, Li F, Zhang L, Yang R, Zhang Z, Zheng D, Gao Y (2009) Dynamic urinary proteomic analysis reveals stable proteins to be potential biomarkers. Proteomics—Clin Appl 3:370–382
13. Utsunomiya T, Ogawa K, Yoshinaga K, Ohta M, Yamashita K, Mimori K, Inoue H, Ezaki T, Yoshikawa Y, Mori M (2005) Clinicopathologic and prognostic values of apolipoprotein D alterations in hepatocellular carcinoma. Int J Cancer 116:105–109