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PROTEOMICS APPROACHES IN THE QUEST OF KIDNEY DISEASE BIOMARKERS

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A b s t r a c t: Proteomics refers to a group of analytical techniques for high throughput protein analysis, providing evidence for protein expression levels, subcellular localization, post-translational modifications and molecular interactions. As such, proteomics has contributed largely to our knowledge regarding molecular mechanisms underlying health and disease and pinpointed potential disease biomarkers. The scope of this review is to briefly introduce the principles of major proteomics techniques employed in biological research, including novel quantitative and molecular imaging mass spectrometry-based platforms. A few examples from the application of these techniques in biomarker discovery for kidney diseases are also provided.

Key words: proteomics**,** kidney disease, biomarker, mass spectrometry, imaging.

Introduction

Proteomics is the large-scale analysis of proteins in biological mixtures. Technological advancements the past years have resulted in a significant increase in the application of proteomics methodologies to answer questions of biological and clinical importance. The scope of this review is to briefly introduce the principles of major proteomics techniques employed in biological research, including novel quantitative and molecular imaging mass spectrometry-based platforms. A few examples from the application of these techniques in understanding renal diseases at the molecular level and discovering biomarkers are also provided.

Proteomics Workflow

The proteomics workflow in brief, consists of two main parts: protein separation, usually conducted by the use of gel-based and/or liquid chromatography-based approaches, and protein identification conducted mainly by mass spectrometry (Figure 1).

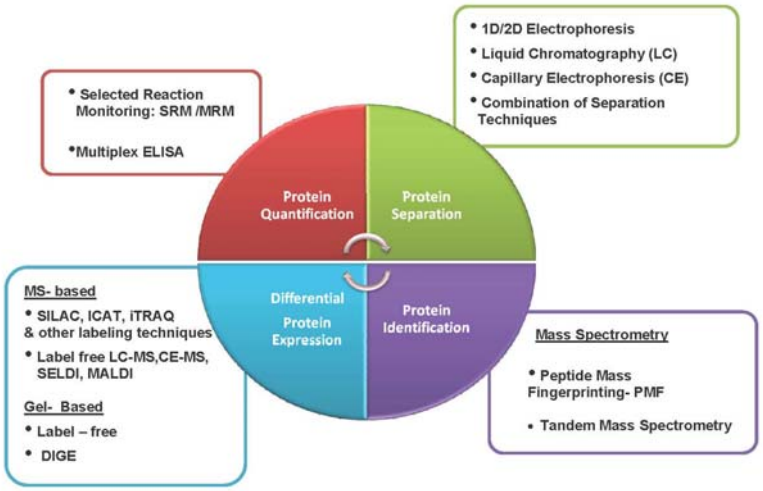

Figure 1 – Common techniques employed for protein separation, identification, relative and absolute quantification. The 3 former are widely used in biomarker discovery with the latter being employed for biomarker verification and validation. SILAC, ICAT and iTRAQ, refer to different labeling techniques commonly used in LC-MS-based proteomics for differential expression analysis.

Abbreviations: MS, Mass spectrometry; PMF, Peptide Mass Fingerprinting; MALDI, Matrix assisted laser/ desorption ionization LC, Liquid Chromatography; CE, Capillary Electrophoresis; SILAC, Stable isotope labeling by amino acids in cell culture; ICAT, Isotope-coded affinity tag; iTRAQ, Isobaric Tag for relative and absolute quantification; SRM /MRM, Selective Reaction Monitoring; ELISA, Enzymed-linked Immunosorbent assay; SELDI, Surface enhanced laser desorption /ionization; DIGE, Differential in-gel electrophoresis.

Protein separation

Gel-based assays have been widely used in proteomics research for protein separation, qualitative and quantitative analysis. Separation is performed based on size on porous gels (most frequently composed of acrylamide). In clas-

sical protocols, proteins are initially denatured and homogeneously charged in the presence of the anionic detergent sodium dodecyl sulphate (SDS); following the application of an electric field, high mass proteins exhibit lower mobility and are retained longer by the polyacrylamide network than smaller proteins, allowing thus for protein separation based on size. Gels of a constant acrylamide concentration or gradient gels may be used allowing respectively the visualization of proteins with a wide spectrum of molecular masses (5–200 kDa), or higher resolution of specific mass ranges [1, 2]. Two-dimensional polyacrylamide gel electrophoresis (2DE) has been the workhorse for numerous proteomics investigations. In this case, besides separation based on size, proteins are additionally separated based on their charge by the use of a technique called isoelectric focussing (IEF) [3]. In both cases of one-dimensional or two-dimensional electrophoresis, visualization of the detected proteins is conducted by a colorimetric assay, quantification by the use of specialized image analysis software and further protein identification by mass spectrometry (described also below [4]**)**. The main advantages of 2DE include high protein resolution e.g. ability to separate up to 10,000 intact protein species [5], including protein isoforms and allowing thus the detection of post-translational modifications [6]. As expected, it also presents several limitations including difficulties in the separation and detection of hydrophobic proteins (such as membrane proteins) as well as highly alkaline or acidic proteins [7]. Additionally, in spite of advancements in image analysis software and automation of protein excision, 2D gel analysis remains labour intensive. Despite these limitations, 2DE is a powerful proteomics technique as it uniquely allows visualization of intact proteins and has been widely used in biomarker discovery and proteome mining applications (e.g., in the analysis of urine proteome in normal and disease states [8]).

A variation of the technique facilitating protein differential expression analysis and quantification, developed over the past few years, is the difference gel electrophoresis (DIGE). This method is based on differential fluorescent labelling of proteins to allow simultaneous separation of the protein groups under comparison on the same gel [9]. To increase comparability of data from different experiments, a control sample labelled by the use of a third label has also been analyzed in every gel separation [10, 11]. In this way, more reliable quantification of protein expression differences and increase in statistical power of observations may be achieved [12].

An alternative approach for protein separation, also widely used in proteomics applications, is liquid chromatography; in this case, proteins are separated based on their physical, chemical and/or immunological properties through a sorbent material [13, 14]. Numerous chromatography schemes can be developed depending on the properties of the latter and the applied binding and elution conditions (e.g. ion-exchange, hydrophobic, metal binding, etc.) [15]. In addition a combination of the above separation schemes (e.g. multi-dimensional

chromatography) may be applied to increase protein resolution [16]. In combination with mass spectrometry, liquid chromatography has been widely used for biomarker discovery and as described below, in biomarker quantification and verification [17–19]. This is a very powerful and comprehensive approach providing higher proteome coverage compared to electrophoretic techniques, nevertheless, due to its frequent combination with protein digestion for further analysis by mass spectrometry, valuable information at the protein level may be lost.

Various separation approaches have been adopted in the past decades for the separation of the low molecular weight proteome (< 20 kD; often referred to as "peptidome"). This includes, capillary electrophoresis in combination to mass spectrometry (CE-MS). In this case, separation is based on the different migration characteristics of the analytes through a liquid filled capillary column in the presence of an electrical field [20]. It has been shown to be a fast and robust separation method providing high resolution analysis of the low MW proteome. On the down-side, due to the limited capacity of the capillaries, only small sample volumes can be loaded [21]. Nevertheless, being a low cost and highly sensitive method, it has been applied extensively in clinical proteomics investigations, in particularly involving the analysis of urine samples [22–24].

The low MW proteome has also been investigated by the use of the SELDI technique. The system consists of chip arrays of various chromatographic properties, (hydrophobic, ion exchange, normal phase, immobilized metal, etc.), where proteins are captured according to their properties. Retained molecules are then washed with buffers of various stringencies and their protein mass information acquired through mass spectrometry [25]. This approach provides a rapid and high-throughput analysis of the peptidome, and has been applied in multiple biomarker discovery investigations; nevertheless its resolving power is significantly lower compared to that of the CE-MS peptidomic profiling [2, 26–28].

Protein identification and quantification by the use of Mass Spectrometry

Regardless of the applied protein separation methodology, protein identification and, in the case of LC and CE, protein quantification is performed by the use of mass spectrometry. This method involves the detection of charged protein molecules and their analysis according to their mass-to-charge (m/z) ratios.

In brief, analysis of a protein molecule (analyte) by mass spectrometry occurs in three steps: a) protein ionization and generation of gas-phase ions; b) ion separation according to mass and c) ion detection. These functions are per-

formed by the three main components of a mass spectrometer: the ion source, the mass analyser and the detector, respectively (reviewed in [29, 30]).

Significant improvements in the last two decades, particularly in the methods of ionization and separation, have established mass spectrometry as the main tool of protein analysis in proteomics investigations. Matrix-assisted laser desorption/ ionization (MALDI) and electrospray ionization (ESI) are the two main ionization methods currently employed in proteomics, relying respectively on protein ionization by laser irradiation assisted by a small organic molecule (matrix) [31], and ionization out of a solution by spraying from a high voltage needle [32]. MALDI and ESI techniques are very powerful allowing study of macromolecules, even protein complexes in the case of ESI. They also exhibit distinct strengths including very efficient coupling of ESI with liquid chromatography separation systems facilitating protein sequencing applications and, in the case of MALDI, higher throughput of analysis and also recent technological developments allowing for the efficient proteomic profiling of histological sections (MALDI-MSI, summarized below [33]).

ESI and MALDI can be coupled to various mass analysers including time-of-flight (TOF), quadrupole (Q), ion traps (QIT), Fourier transform ion cyclotron (FT-MS), recently developed orbitraps (Orbi), and in cases combinations thereof in the form of hybrid mass spectrometry systems. A detailed presentation of the principles of operation of the different mass analysers would be out of the scope of this review, and the interested reader may refer to [29, 34] on this issue. As expected, different mass analysers differ in the accuracy, sensitivity and resolution in mass detection, ease of operation, type of allowed associated applications (e.g. identification, determination of post-translational modifications, quantification) as well as associated cost [29, 34, 35].

Protein identification by the use of mass spectrometry relies mainly on enzymatic protein fragmentation and subsequent determination of the generated peptide masses by MS (bottom-up approach). In brief, in a process called peptide mass fingerprinting (PMF) or peptide mass mapping, this experimental peptide map (e.g. collection of peptide masses generated following proteolysis), is compared to the theoretical peptide maps of known proteins, by the use of mathematical tools called search engines [36]. The output of such analysis is protein identification on a probabilistic basis based on percent match between the experimental and theoretical peptide maps. To verify results of peptide map fingerprinting and obtain sequencing data, Tandem mass spectrometry is applied [37]. In this case peptide fragments are further fragmented frequently by CID (collision induced dissociation) or ETD (electron transfer dissociation) within the mass spectrometer. CID employs neutral gas molecules such as helium, nitrogen, or argon which, upon collision with the protein fragments induce their further

fragmentation [34]; in the case of ETD fluoranthene radicals are applied as electron donors to destabilize peptide ions. ETD is widely used for the analysis of posttranslational modifications [38, 39]. In the case of tandem MS data analysis, and similar to PMF, identification is finally obtained by the application of search engines comparing the results of the experimental analysis to *in silico* protein data [37].

Besides protein identification, mass spectrometry may also provide information on the protein quantity within a mixture. Usually this is confined to relative protein quantities (e.g. in relation to other components in the mixture) [24, 40, 41], whereby by comparison to standards, measurements on absolute quantities may also be obtained [42]. MRM or Multiple Reaction Monitoring involves the absolute quantitative analysis of biomolecules by monitoring and quantifying their fragmentation products in comparison to standards [43, 44]. Usually triple quadrupole mass analysers are employed for such applications, providing high selectivity in ion detection. Even though technical hurdles exist, (such as the need for prototype-unique peptides per protein, and in cases that need protein enrichment prior to MS, etc.), MRM is currently considered a highly sensitive, and selective method for protein quantification presenting the additional advantages of multiplexing and high-throughput [43, 45]. As such, MRM is considered a powerful and very promising alternative to immunoassays for biomarker quantification and validation [46, 47].

Imaging MS

The aforementioned techniques are regularly applied for the analysis of protein extracts. Imaging mass spectrometry (IMS) is a powerful technique that combines the detection of biomolecules achieved by mass spectrometry with microscopic imaging capabilities [33]. Direct analysis of intact tissues of biological and clinical interest without applying homogenization and separation stages has been shown to successfully preserve the spatial distribution of molecules within the tissue. As such, IMS enables the detection and identification of several biological molecules, such as proteins, peptides, lipids and metabolites in a single measurement while maintaining their spatial localization within the tissue.

In most applications, MALDI-MS is employed. In brief, a matrix is uniformly applied over the surface of thin tissue sections (~10 μm) by automatic deposition with robotic liquid dispensing devices, followed by irradiation of the sample in an ordered array and desorption of the proteins (or other selected biomolecules) from specific spots or pixels. Spectra are acquired from each discrete spot/pixel that corresponds to a specific X,Y coordinate location on the tissue section. The intensity of each m/z value can be depicted as a 2D ion

density map of the tissue section [48]. The achieved lateral resolution is approximately 10–50 μm [49, 50].

This technology has been shown to have a great potential for the discovery and identification of biomarkers [51]. Since the initial application of MALDI IMS to tissue sections in 1997 [52], this technology has been successfully used in the analysis of a wide variety of normal and diseased tissues. The study of cancer tissues has been a major focus of IMS for protein identification, in order to identify biomarkers that can define grade classifications of cancer biopsies and help in the choice of the appropriate treatment [48, 52–54]. In addition, IMS has been utilized for the determination of metabolite and drug distribution in whole animal sections [55, 56] or tissue sections from particular organs [57, 58].

Collectively these initial data support that imaging-MS could contribute significantly to our knowledge of the molecular changes associated with distinct tissue compartments during disease development. Technological hurdles exist and are related to needed improvements in the overall signal detection, including the ability to detect low abundant molecules as well as difficulties in the identification (e.g. determination of amino acid sequence) of selected molecules. Nevertheless, the technique of imaging mass spectrometry is continuously becoming more sensitive as high quality MALDI-IMS experiments can be performed with the modern mass spectrometers that provide improved speed, higher mass and spatial resolution [59, 60].

Proteomics technologies in renal disease research

The advances in the mass spectrometric platforms have made these proteomic methods very popular among the scientific community and clinicians who are involved in kidney diseases. There are many clinical applications for the discovery of potential protein biomarkers for renal diseases and diabetic nephropathy in which 2DE, LC, CE – in combination to Mass Spectrometry have been used (for example for DN [61, 62, 63], Kidney Injury [64–65], Fanconi Syndrome [67, 68], IgA Nephropathy [69], Congenital Obstructive Nephropathy [70, 71] and Vesicoureteral Reflux [72]. There have been various recent reviews summarizing biomarker findings on these diseases [61–74] and, as such, a detailed description of biomarker findings would be out of the scope of this review. Table 1 summarizes some of the findings that are directly linked to disease pathophysiology mechanisms, and a few additional examples are provided below, so as to make more clear to the reader the applicability of these proteomics applications in Chronic Kidney Disease (CKD) biomarker and molecular pathology research.

Table 1

Some of the known renal biomarkers as related to specific pathophysiological mechanism

Biomarkers associated with renal functional mechanisms

Biological Mechanism	Biomarker	Biological Fluid	Related Kidney Disease	Studies
Glomerular Injury	Nephrin	Urine	Diabetic Nephropathy, Active Lupus Nephritis	Wang et al. 2007 [91]
	Podocin	Urine	Diabetic Nephropathy, Active Lupus Nephritis	Wang et al. 2007 [91]
	Podocalyxin		IgA Nephropathy, Lupus Nephritis	Kanno et al. 2003 [92]
Tubulointerstitial Injury	NGAL	Serum and Urine	Early biomarker of AKI	Bolignano et al. 2008 [93]
	KIM-1	Urine	AKI to CKD transition	Ko et al. 2010 [94]
	NAG	Urine	Proximal Tubular Damage	Bosomworth et al. 1999 [95]
Kidney Function (GFR)	Cystatin c	Serum	CKD progression	Spanaus et al. 2010 [96]
Fibrosis	TGF-β1	Urine	Diabetic Nephropathy, Glomeruloronephritis	Tonelli et al. 2005 [97]
Cardiovascular Dysfunction	BNP	Plasma	CKD trajecteory	Sakuma et al. 2010 [98]
	ANP	Plasma	CKD trajecteory	Dieplinger et al. 2009 [99]
Endothelial Dysfunction	ADMA	Plasma	End stage Kidney Disease	Ravani et al. 2005 [100]
Oxidative Stress	Ox-LDL	Plasma /Serum	CKD, Endothelial injury, Inflammation	Holvoet et al. 1996 [101]
	Urinary 8-hydroxydeoxy guanosine	Plasma /Serum	Early CKD, Diabetic Nephropathy	Dincer et al. 2008 [102]
Inflammation	CRP	Plasma	CKD, Inflammation	Fakhrzadeh et al. 2009 [103]
	IL18	Urine	Kidney Tubular Injury, AKI from ischemia	Keller et al. 2010 [104]
	TNF-receptor II	Plasma	CKD	Tonelli et al. 2005 [105]
Metabolic Disorders	Adiponectin		Early Detection of CKD	Saraheimo et al. 2008 [106]
	FGF-23	Serum	CKD, ESDR	Westerberg et al. 2007 [107]
	ApoA-IV		ESDR, Early CKD	Kronenberg et al. 2002 [2]

CKD is the final outcome of many different renal diseases; some of them are of renal etiology (e.g. glomerulonephritis, interstitial renal fibrosis) and others have a systemic etiology (e.g. diabetes, hypertension). CKD is a medical problem affecting many individuals in all societies around the world; Studies from Europe [76] and the United States [77] have indicated that about 10–13% of the whole adult population is suffering from a CKD stage. Furthermore, about 1% of those individuals can be classified in stage 5, which indicates End-Stage Renal Disease (ESRD). ESRD patients represent a major socio-economic problem with severe implications for individuals, families and societies, since they need replacement therapy.

From the above, it is obvious that careful screening of large parts of the population may have major beneficial effects, since early therapeutic intervenetion could reduce the rate of the progression of the disease, or even halt it and, hopefully, with future therapies regression could be an attainable goal. Such screenings are currently based on assessment of albuminuria and glomerular filtration rate (GFR). Although these provide a good estimate of renal function, they do not provide an accurate view of the condition of the renal parenchyma, where major alterations are silent for many years. Therefore, there is an urgent need for novel and more precise markers of renal damage. Irrespective of the initiating factor and disease that leads to the renal damage, the common anatomical characteristic is the development of fibrosis. Fibrosis is defined as the accumulation of an extracellular matrix that is pathological both in amount (excessive) and in nature (different macromolecules). Consequently, finding early markers for the development of renal fibrosis is of paramount importance, because they can alert us about a condition that goes asymptomatic for many years. Such advanced knowledge can lead to early and more effective therapeutic interventions.

In the process of renal fibrosis, we have at the beginning the initiating factors, which can be either physical, such as an increase in the vascular or the tubular pressure or chemical, such as an increase in glucose concentration (in diabetes) and/or in albumin concentration in the tubular lumen (in many proteinuric conditions). These conditions are sensed by the renal cells; all types of renal cells have been implicated as playing a role in the development of fibrosis, namely the epithelial cells, endothelial cells, pericytes, mesangial cells and fibroblast. The early response of the affected cells is the secretion of cytokines and chemokines such as TGF-β, PDGF, bFGF, CTGF, endothelin, osteopontin and many others. As a result of local accumulation and action of these early factors, critical late products are secreted which represent the hallmark of a full-blown fibrosis; these include structural extracellular macromolecules (collagen types, fibronectin, elastin, tubulointerstitial nephritis antigen, etc.), crosslinking enzymes (transglutaminase), enzymes affecting degradation (MMPs, plasmin, elastase, etc.) and macromolecules affecting their enzymatic activity (TIPMs,

plasminogen activator and its inhibitor, components of the kinin-kallikrein system, etc.) [78].

To what extent have new proteomic approaches helped in elucidating these processes and finding new markers? We will review in brief the existing literature, focussing on two types of glomerular pathologies where there is a prominent fibrotic component: diabetic nephropathy (DN) and focal segmental glomerulosclerosis (FSGS).

DN is responsible for the majoity of patients in ESRD. Fibrosis is observed in all renal compartments, especially in the mesangial space, but also in the tubulointerstitial compartment. Studies analysing the urinary proteome at early stages have uncovered lists of biomarkers that distinguish patients with normoalbuminuria and nephropathy; many of the biomarkers were fragments of type I collagen [79]. In type 1 diabetics followed for a 10 year period distinguishing them in those with intact and those with affected renal function, it was found that altered levels of peptides from six different proteins (Collagen IV, collagen V, tenascin decreased, whereas inositol pentakisphosphate 2-kinase, ZO-3, FAT tumour-suppressor) were detected in these two groups [80]. Proteomic studies of urine samples from advanced stages of DN showed that most of the differentially expressed proteins were abundant plasma proteins, which is not surprising, given the increased permeability of the glomerular filtration barrier [9, 81]. It is worth mentioning that in one study the protein alpha 1 antitryspin was found highly up-regulated in 2-D gels and this finding was further confirmed by ELISA and by immunostaining of diabetic kidneys [9]. However, a lot needs to be done in order to extend and enrich our knowledge regarding specific markers for fibrosis during the course of the development of DN in humans. A few studies exist in animal models [82, 83], where differential expression of specific proteins has been discovered and documented. The list includes elastase, elastase inhibitor, tubulointerstitial nephritis antigen and heparin sulphate proteoglycan-2 (for a short review see [78]). However, caution should be exerted in extrapolating results from animal models of diabetic nephropathy to human patients and further work is needed.

FSGS is a frequent initial condition leading to CKD both in adults and children and approximately 20% of those patients are at risk of developing CKD. According to the Columbia classification [84], several types of different morphological alterations/glomerular injuries are observed and these can imply differences in etiology or in pathogenetic mechanisms. An early study with a limited number of urine samples has identified a specific pattern different from the pattern obtained from "normal" urine samples [85]. No further studies focused on FSGS exist at the moment. Studies with animal models of FSGS exist [86, 87] and have suggested that certain macromolecules (thymosin b4, collagen IV, ADAM32, cerberus, tomoregulin) may be markers of the diseases. Again,

animal studies, especially in cases where no animal model exists for FSGS, should be treated with caution regarding extrapolation of their results.

Another approach in order to better understand the fibrotic process and gain more information about macromolecules involved in it would be to explore the prognostic/diagnostic value of members of lists produced from proteomic studies of fibrotic events not confined to the glomerulus. As an example, our group has examined the differential expression of proteins in the renal parenchyma in the Unilateral Ureteric Obstruction (UUO) model of rodents. This is a convenient and commonly used animal model of fibrosis, since it recapitulates in two weeks the fibrotic processes that take 10–20 years to develop in human renal diseases. Our studies have led to the production of lists of specific macromolecules differentially regulated during early and late phases of the development of fibrosis in the UUO model [88]. We have been focussing on two of these proteins, calreticulin and transgelin. Although transgelin was not observed up-regulated in the animal model inside the glomerulus, when we examined several sections from human renal biopsies, it was clearly found up-regulated in certain glomerulopahies. Furthermore, expression of transgelin in the glomerulus was in certain cases co-distributed with that of α-SMA (the classical marker for activated myofibroblasts) but this was not always the case, suggesting the existence of subpopulations of fibroblasts [89].

In parallel to these classical approaches, applications of MALDI-Imaging in the investigation of kidney diseases have also been reported. Besides investigation of renal malignancies [90], MALDI-Imaging methodologies have been also successfully used to study drug-induced renal toxicity [91]. In this study, MALDI-Imaging has been applied to rat kidney in order to reveal the proteomic changes induced by gentamicin, a well-known nephrotoxicant that could contribute to the etiology of tubule damage. Structural and functional information of the cortex of affected kidneys revealed transthyretin as a toxicity marker [56].

It is obvious that although proteomic technologies and approaches are extremely promising towards the goal of a better understanding of renal pathology and improving our prognostic/diagnostic capacities, major gaps still exist in our knowledge. Large validation studies are needed to verify existing biomarker findings while at the same time further targeted, hypothesis-driven molecular investigations are required to obtain a better understanding of the underlying molecular mechanisms of disease.

Outlook

The application of Proteomics technologies has provided valuable information on deciphering mechanisms of disease pathophysiology and indicated potential disease biomarkers. Due to the vast complexity of the proteome, infor-

mation received by the different methods and platforms is highly complementary and its integration will be ultimately needed to increase comprehendsiveness. In addition, powerful mass spectrometry-based techniques have been developed in the last few years allowing comprehensive profiling of body fluids and also, in a high throughput, in a sensitive and selective manner, protein quantification. In the case of renal diseases more specifically, we foresee the following important contributions from proteomics research:

1) developing the precise proteome of distinct renal compartments (glomeruli vs. tubules, cortex vs. medulla);

2) deciphering the proteome of distinct functional units (for example, the juxtaglomerular apparatus);

3) studying the proteome of different cell types inside the same compartment (for example podocytes vs. parietal epithelial cells);

4) ultimately being able to generate the proteomic profile of different subtypes of phenotypically similar cells.

Our progress towards these goals in the near future will certainly allow a better understanding of renal pathology, a reclassification of renal diseases and ultimately a clear definition of better therapeutic targets.

REFERENCES

1. Chakavarti B, Chakavarti D. Electrophoretic separation of proteins. J Vis Exp. 2008; 16: 758.

2. Vlahou A, Fountoulakis M. Proteomic approaches in the search for disease biomarkers. J Chromatogr B Analyt Technol Biomed Life Sci. 2005; 814(1): 11–19.

3. Friedman DB, Hoving S, Westermeier R. Isoelectric focusing and two-dimensional gel electrophoresis. Methods Enzymol. 2009; 463: 515–540.

4. Meleady P. 2D gel electrophoresis and mass spectrometry identification and analysis of proteins. Methods Mol Biol. 2011; 784: 123–137.

5. Marcus K, Joppich C, May C, et al. High-resolution 2DE. Methods Mol Biol. 2009; 519: 221–240.

6. Barnouin K. Two-dimensional gel electrophoresis for analysis of protein complexes. Methods Mol Biol. 2004; 261: 479–498.

7. Rabilloud T, Chevallet M, Luche S, Lelong C. Two-dimensional gel electrophoresis in proteomics: Past, present and future. J Proteomics. 2010; 73(11): 2064–2077.

8. Candiano G, Santucci L, Petretto A, et al. 2D-electrophoresis and the urine proteome map: where do we stand? J Proteomics. 2010; 73(5): 829–844.

9. Sharma K, Lee S, Han S, et al. Two-dimensional fluorescence difference gel electrophoresis analysis of the urine proteome in human diabetic nephropathy. Proteomics. 2005; 5(10): 2648–2655.

10. Sapra R. The use of difference in-gel electrophoresis for quantitation of protein expression. Methods Mol Biol. 2009; 492: 93–112.

11. Minden JS, Dowd SR, Meyer HE, Stuhler K. Difference gel electrophoresis. Electrophoresis. 2009; 30 Suppl 1(S1): 56–161.

12. Timms JF, Cramer R. Difference gel electrophoresis. Proteomics. 2008; 8(23–24): 4886–4897.

13. Ewles M, Goodwin L. Bioanalytical approaches to analyzing peptides and proteins by LC--MS/MS. Bioanalysis. 2011; 3(12): 1379–1397.

14. Xu RN, Fan L, Rieser MJ, El-Shourbagy TA. Recent advances in high throughput quantitative bioanalysis by LC-MS/MS. J Pharm Biomed Anal. 2007; 44(2): 342–355.

15. Halquist MS, Thomas Karnes H. Quantitative liquid chromatography tandem mass spectrometry analysis of macromolecules using signature peptides in biological fluids. Biomed Chromatogr. 2011; 25(1–2): 47–58.

16. Gao M, Qi D, Zhang P, Deng C, Zhang X. Development of multidimensional liquid chromatography and application in proteomic analysis. Expert Rev Proteomics. 2010; 7(5): 665–678.

17. Qian WJ, Jacobs JM, Liu T, Camp DG, 2nd, Smith RD. Advances and challenges in liquid chromatography-mass spectrometry-based proteomics profiling for clinical applications. Mol Cell Proteomics. 2006; 5(10): 1727–1744.

18. Seliger B, Dressler SP, Lichtenfels R, Kellner R. Candidate biomarkers in renal cell carcinoma. Proteomics. 2007; 7(24): 4601–4612.

19. Shushan B. A review of clinical diagnostic applications of liquid chromatography-tandem mass spectrometry. Mass Spectrom Rev. 2010; 29(6): 930–944.

20. Thormann W. Capillary electrophoretic separations. Methods Biochem Anal. 2011; 54: 451–485.

21. Mischak H, Coon JJ, Novak J, Weissinger EM, Schanstra JP, Dominiczak AF. Capillary electrophoresis-mass spectrometry as a powerful tool in biomarker discovery and clinical diagnosis: an update of recent developments. Mass Spectrom Rev. 2009; 28(5): 703–724.

22. Albalat A, Mischak H, Mullen W. Clinical application of urinary proteomics/peptidomics. Expert Rev Proteomics. 2011; 8(5): 615–629.

23. Gamagedara S, Ma Y. Biomarker analysis for prostate cancer diagnosis using LC-MS and CE-MS. Bioanalysis. 2011; 3(18): 2129–2142.

24. Siwy J, Mullen W, Golovko I, Franke J, Zurbig P. Human urinary peptide database for multiple disease biomarker discovery. Proteomics Clin Appl. 2011; 5(5–6): 367–374.

25. Poon TC. Opportunities and limitations of SELDI-TOF-MS in biomedical research: practical advices. Expert Rev Proteomics. 2007; 4(1): 51–65.

26. De Bock M, de Seny D, Meuwis MA, et al. Challenges for biomarker discovery in body fluids using SELDI-TOF-MS. J Biomed Biotechnol. 2010; 2010: 906082.

27. Neuhoff N, Kaiser T, Wittke S, et al. Mass spectrometry for the detection of differentially expressed proteins: a comparison of surface-enhanced laser desorption/-ionization and capillary electrophoresis/mass spectrometry. Rapid Commun Mass Spectrom. 2004; 18(2): 149–156.

28. Whelan LC, Power KA, McDowell DT, Kennedy J, Gallagher WM. Applications of SELDI-MS technology in oncology. J Cell Mol Med. 2008; 12(5A): 1535–1547.

29. Aebersold R, Mann M. Mass spectrometry-based proteomics. Nature. 2003; 422(6928): 198–207.

30. Guerrera IC, Kleiner O. Application of mass spectrometry in proteomics. Biosci Rep. 2005; 25(1–2): 71–93.

31. Karas M. Matrix-assisted laser desorption ionization MS: a progress report. Biochem Soc Trans. 1996; 24(3): 897–900.

32. Smith RD, Loo JA, Edmonds CG, Barinaga CJ, Udseth HR. New developments in biochemical mass spectrometry: electrospray ionization. Anal Chem. 1990; 62(9): 882–899.

33. Francese S, Dani FR, Traldi P, Mastrobuoni G, Pieraccini G, Moneti G. MALDI mass spectrometry imaging, from its origins up to today: the state of the art. Comb Chem High Throughput Screen. 2009; 12(2): 156–174.

34. Canas B, Lopez-Ferrer D, Ramos-Fernandez A, Camafeita E, Calvo E. Mass spectrometry technologies for proteomics. Brief Funct Genomic Proteomic. 2006; 4(4): 295–320.

35. Khalsa-Moyers G, McDonald WH. Developments in mass spectrometry for the analysis of complex protein mixtures. Brief Funct Genomic Proteomic. 2006; 5(2): 98–111.

36. Thiede B, Hohenwarter W, Krah A, et al. Peptide mass fingerprinting. Methods. 2005; 35(3): 237–247.

37. Edwards NJ. Protein identification from tandem mass spectra by database searching. Methods Mol Biol. 2011; 694(119–138).

38. Wiesner J, Premsler T, Sickmann A. Application of electron transfer dissociation (ETD) for the analysis of posttranslational modifications. Proteomics. 2008; 8(21): 4466–4483.

39. Pittenauer E, Allmaier G. High-energy collision induced dissociation of biomolecules: MALDI-TOF/RTOF mass spectrometry in comparison to tandem sector mass spectrometry. Comb Chem High Throughput Screen. 2009; 12(2): 137–155.

40. Brewis IA, Brennan P. Proteomics technologies for the global identification and quantification of proteins. Adv Protein Chem Struct Biol. 2010; 80: 1–44.

41. Zhu W, Smith JW, Huang CM. Mass spectrometry-based label-free quantitative proteomics. J Biomed Biotechnol. 2010; 2010(840518).

42. Bantscheff M, Schirle M, Sweetman G, Rick J, Kuster B. Quantitative mass spectrometry in proteomics: a critical review. Anal Bioanal Chem. 2007; 389(4): 1017–1031.

43. Kitteringham NR, Jenkins RE, Lane CS, Elliott VL, Park BK. Multiple reaction monitoring for quantitative biomarker analysis in proteomics and metabolomics. J Chromatogr B Analyt Technol Biomed Life Sci. 2009; 877(13): 1229–1239.

44. Gallien S, Duriez E, Domon B. Selected reaction monitoring applied to proteomics. J Mass Spectrom. 2011; 46(3): 298–312.

45. Yocum AK, Chinnaiyan AM. Current affairs in quantitative targeted proteomics: multiple reaction monitoring-mass spectrometry. Brief Funct Genomic Proteomic. 2009; 8(2): 145–157.

46. Elschenbroich S, Kislinger T. Targeted proteomics by selected reaction monitoring mass spectrometry: applications to systems biology and biomarker discovery. Mol Biosyst. 2011; 7(2): 292–303.

47. Selevsek N, Matondo M, Sanchez Carbayo M, Aebersold R, Domon B. Systematic quantification of peptides/proteins in urine using selected reaction monitoring. Proteomics. 2011; 11(6): 1135–1147.

48. Seeley EH, Caprioli RM. Molecular imaging of proteins in tissues by mass spectrometry. Proc Natl Acad Sci USA. 2008; 105(47): 18126–18131.

49. Yang J, Caprioli RM. Matrix sublimation/recrystallization for imaging proteins by mass spectrometry at high spatial resolution. Anal Chem. 2011; 83(14): 5728–5734.

50. Walch A, Rauser S, Deininger SO, Hofler H. MALDI imaging mass spectrometry for direct tissue analysis: a new frontier for molecular histology. Histochem Cell Biol. 2008; 130(3): 421–434.

51. Cazares LH, Troyer DA, Wang B, Drake RR, Semmes OJ. MALDI tissue imaging: from biomarker discovery to clinical applications. Anal Bioanal Chem. 2011; 401(1): 17–27.

52. Caprioli RM, Farmer TB, Gile J. Molecular imaging of biological samples: localization of peptides and proteins using MALDI-TOF MS. Anal Chem. 1997; 69(23): 4751–4760.

53. Rauser S, Marquardt C, Balluff B, et al. Classification of HER2 receptor status in breast cancer tissues by MALDI imaging mass spectrometry. J Proteome Res. 2010; 9(4): 1854–1863.

54. Schwartz SA, Weil RJ, Thompson RC, et al. Proteomic-based prognosis of brain tumor patients using direct-tissue matrix-assisted laser desorption ionization mass spectrometry. Cancer Res. 2005; 65(17): 7674–7681.

55. Khatib-Shahidi S, Andersson M, Herman JL, Gillespie TA, Caprioli RM. Direct molecular analysis of whole-body animal tissue sections by imaging MALDI mass spectrometry. Anal Chem. 2006; 78(18): 6448–6456.

56. Meistermann H, Norris JL, Aerni HR, et al. Biomarker discovery by imaging mass spectrometry: transthyretin is a biomarker for gentamicin-induced nephrotoxicity in rat. Mol Cell Proteomics. 2006; 5(10): 1876–1886.

57. Hsieh Y, Chen J, Korfmacher WA. Mapping pharmaceuticals in tissues using MALDI imaging mass spectrometry. J Pharmacol Toxicol Methods. 2007; 55(2): 193–200.

58. Reyzer ML, Caprioli RM. MALDI-MS-based imaging of small molecules and proteins in tissues. Curr Opin Chem Biol. 2007; 11(1): 29–35.

59. Zaima N, Hayasaka T, Goto-Inoue N, Setou M. Matrix-assisted laser desorption/ionization imaging mass spectrometry. Int J Mol Sci. 2010; 11(12): 5040–5055.

60. McDonnell LA, Corthals GL, Willems SM, van Remoortere A, van Zeijl RJ, Deelder AM. Peptide and protein imaging mass spectrometry in cancer research. J Proteomics. 2010; 73(10): 1921–1944.

61. Ben Ameur R, Molina L, Bolvin C, et al. Proteomic approaches for discovering biomarkers of diabetic nephropathy. Nephrol Dial Transplant. 2010; 25(9): 2866–2875.

62. Merchant ML, Klein JB. Proteomics and diabetic nephropathy. Semin Nephrol. 2007; 27(6): 627–636.

63. Mischak H, Rossing P. Proteomic biomarkers in diabetic nephropathy-reality or future promise? Nephrol Dial Transplant. 2010; 25(9): 2843–2845.

64. Devarajan P. Biomarkers for the early detection of acute kidney injury. Curr Opin Pediatr. 2011; 23(2): 194–200.

65. Devarajan P, Krawczeski CD, Nguyen MT, Kathman T, Wang Z, Parikh CR. Proteomic identification of early biomarkers of acute kidney injury after cardiac surgery in children. Am J Kidney Dis. 2010; 56(4): 632–642.

66. Siew ED, Ware LB, Ikizler TA. Biological markers of acute kidney injury. J Am Soc Nephrol. 2011; 22(5): 810–820.

67. Caubet C, Lacroix C, Decramer S, et al. Advances in urinary proteome analysis and biomarker discovery in pediatric renal disease. Pediatr Nephrol. 2010; 25(1): 27–35.

68. Wu J, Chen YD, Gu W. Urinary proteomics as a novel tool for biomarker discovery in kidney diseases. J Zhejiang Univ Sci B. 2010; 11(4): 227–237.

69. Julian BA, Wittke S, Haubitz M, et al. Urinary biomarkers of IgA nephropathy and other IgA-associated renal diseases. World J Urol. 2007; 25(5): 467–476.

70. Chevalier RL. Biomarkers of congenital obstructive nephropathy: past, present and future. J Urol. 2004; 172(3): 852–857.

71. Decramer S, Zurbig P, Wittke S, Mischak H, Bascands JL, Schanstra JP. Identification of urinary biomarkers by proteomics in newborns: use in obstructive nephropathy. Contrib Nephrol. 2008; 160: 127–141.

72. Lee RS. Biomarkers for pediatric urological disease. Curr Opin Urol. 2009; 19(4): 397–401.

73. Goo YA, Tsai YS, Liu AY, Goodlett DR, Yang CC. Urinary proteomics evaluation in interstitial cystitis/painful bladder syndrome: a pilot study. Int Braz J Urol. 2010; 36(4): 464–478.

74. Liu BC, Ehrlich JR. Proteomics approaches to urologic diseases. Expert Rev Proteomics. 2006; 3(3): 283–296.

75. de Jong PE, van der Velde M, Gansevoort RT, Zoccali C. Screening for chronic kidney disease: where does Europe go? Clin J Am Soc Nephrol. 2008; 3(2): 616–623.

76. Coresh J, Selvin E, Stevens LA, et al. Prevalence of chronic kidney disease in the United States. JAMA.2007; 298(17): 2038–2047.

77. Klein J, Kavvadas P, Prakoura N, et al. Renal fibrosis: insight from proteomics in animal models and human disease. Proteomics. 2011; 11(4): 805–815.

78. Rossing K, Mischak H, Dakna M, et al. Urinary proteomics in diabetes and CKD. J Am Soc Nephrol. 2008; 19(7): 1283–1290.

79. Merchant ML, Perkins BA, Boratyn GM, et al. Urinary peptidome may predict renal function decline in type 1 diabetes and microalbuminuria. J Am Soc Nephrol. 2009; 20(9): 2065–2074.

80. Rao PV, Lu X, Standley M, et al. Proteomic identification of urinary biomarkers of diabetic nephropathy. Diabetes Care. 2007; 30(3): 629–637.

81. Gong D, Chen X, Middleditch M, et al. Quantitative proteomic profiling identifies new renal targets of copper(II)-selective chelation in the reversal of diabetic nephropathy in rats. Proteomics. 2009; 9(18): 4309–4320.

82. Thongboonkerd V, Barati MT, McLeish KR, et al. Alterations in the renal elastin-elastase system in type 1 diabetic nephropathy identified by proteomic analysis. J Am Soc Nephrol. 2004; 15(3): 650–662.

83. D'Agati VD, Fogo AB, Bruijn JA, Jennette JC. Pathologic classification of focal segmental glomerulosclerosis: a working proposal. Am J Kidney Dis. 2004; 43(2): 368–382.

84. Weissinger EM, Wittke S, Kaiser T, et al. Proteomic patterns established with capillary electrophoresis and mass spectrometry for diagnostic purposes. Kidney Int. 2004; 65(6): 2426–2434.

85. Shui HA, Huang TH, Ka SM, Chen PH, Lin YF, Chen A. Urinary proteome and potential biomarkers associated with serial pathogenesis steps of focal segmental glomerulosclerosis. Nephrol Dial Transplant. 2008; 23(1): 176–185.

86. Xu BJ, Shyr Y, Liang X, et al. Proteomic patterns and prediction of glomerulosclerosis and its mechanisms. J Am Soc Nephrol. 2005; 16(10): 2967–2975.

87. Kypreou KP, Kavvadas P, Karamessinis P, et al. Altered expression of calreticulin during the development of fibrosis. Proteomics. 2008; 8(12): 2407–2419.

88. Gerolymos M, Karagianni F, Papasotiriou M, et al. Expression of transgelin in human glomerulonephritis of various etiology. Nephron Clin Pract. 2011; 119(1): c74–82.

89. Oppenheimer SR, Mi D, Sanders ME, Caprioli RM. Molecular analysis of tumor margins by MALDI mass spectrometry in renal carcinoma. J Proteome Res. 2010; 9(5): 2182–2190.

90. Herring KD, Oppenheimer SR, Caprioli RM. Direct tissue analysis by matrix-assisted laser desorption ionization mass spectrometry: application to kidney biology. Semin Nephrol. 2007; 27(6): 597–608.

91. Wang G, Lai FM, Tam LS, et al. Messenger RNA expression of podocyte-associated molecules in urinary sediment of patients with lupus nephritis. J Rheumatol. 2007; 34(12): 2358–2364.

92. Kanno K, Kawachi H, Uchida Y, Hara M, Shimizu F, Uchiyama M. Urinary sediment podocalyxin in children with glomerular diseases. Nephron Clin Pract. 2003; 95(3): c91–99.

93. Bolignano D, Donato V, Coppolino G, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a marker of kidney damage. Am J Kidney Dis. 2008; 52(3): 595–605.

94. Ko GJ, Grigoryev DN, Linfert D, et al. Transcriptional analysis of kidneys during repair from AKI reveals possible roles for NGAL and KIM-1 as biomarkers of AKI-to-CKD transition. Am J Physiol Renal Physiol. 2010; 298(6): F1472–1483.

95. Bosomworth MP, Aparicio SR, Hay AW. Urine N-acetyl-beta-D-glucosaminidase – a marker of tubular damage? Nephrol Dial Transplant. 1999; 14(3): 620–626.

96. Spanaus, KS, et al. Serum creatinine, cystatin C, and beta-trace protein in diagnostic staging and predicting progression of primary nondiabetic chronic kidney disease. Clin Chem. 2010; 5(56): 740.

97. Sakuma M, Nakamura M, Tanaka F, et al. Plasma B-type natriuretic peptide level and cardiovascular events in chronic kidney disease in a community-based population. Circ J. 2010; 74(4): 792–797.

98. Tonelli M, Sacks F, Pfeffer M, Jhangri GS, Curhan G. Biomarkers of inflammation and progression of chronic kidney disease. Kidney Int. 2005; 68(1): 237–245.

99. Dieplinger B, Mueller T, Kollerits B, et al. Pro-A-type natriuretic peptide and pro-adrenomedullin predict progression of chronic kidney disease: the MMKD Study. Kidney Int. 2009; 75(4): 408–414.

100. Ravani P, Tripepi G, Malberti F, Testa S, Mallamaci F, Zoccali C. Asymmetrical dimethylarginine predicts progression to dialysis and death in patients with chronic kidney disease: a competing risks modeling approach. J Am Soc Nephrol. 2005; 16(8): 2449–2455.

101. Holvoet P, Donck J, Landeloos M, et al. Correlation between oxidized low density lipoproteins and von Willebrand factor in chronic renal failure. Thromb Haemost. 1996; 76(5): 663–669.

102. Dincer Y, Sekercioglu N, Pekpak M, Gunes KN, Akcay T. Assessment of DNA oxidation and antioxidant activity in hypertensive patients with chronic kidney disease. Ren Fail. 2008; 30(10): 1006–1011.

103. Fakhrzadeh H, Ghaderpanahi M, Sharifi F, Badamchizade Z, Mirarefin M, Larijani B. Increased risk of chronic kidney disease in elderly with metabolic syndrome and high levels of C-reactive protein: Kahrizak Elderly Study. Kidney Blood Press Res. 2009; 32(6): 457–463.

104. Keller C, Katz R, Sarnak MJ, et al. Inflammatory biomarkers and decline in kidney function in the elderly: the Cardiovascular Health Study. Nephrol Dial Transplant. 2010; 25(1): 119–124.

105. Saraheimo M, Forsblom C, Thorn L, et al. Serum adiponectin and progression of diabetic nephropathy in patients with type 1 diabetes. Diabetes Care. 2008; 31(6): 1165–1169.

106. Westerberg PA, Linde T, Wikstrom B, Ljunggren O, Stridsberg M, Larsson TE. Regulation of fibroblast growth factor-23 in chronic kidney disease. Nephrol Dial Transplant. 2007; 22(11): 3202–3207.

107. Kronenberg F, Kuen E, Ritz E, et al. Apolipoprotein A-IV serum concentrations are elevated in patients with mild and moderate renal failure. J Am Soc Nephrol. 2002; 13(2): 461–469.

Р е з и м е

ПРИСТАПИТЕ НА ПРОТЕОМИКАТА ВО ПОТРАГАТА ПО БИОМАРКЕРИ ЗА БОЛЕСТИТЕ НА БУБРЕЗИТЕ

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А п с т р а к т: Протеомиката се однесува на група на аналитички техники за високата анализа на протеините, обезбедување податоци за нивоата на протеинска експресија, субклеточна локализација, посттранслациски модификации и молекуларни интеракции. Како таква, протеомиката придонесла многу за нашите сознанија за молекуларни механизми кои се во основата на здравјето и болеста и ги одредила потенцијалните биомаркери на болестите. Опсегот на овој преглед е накратко да се воведат принципите на главните техники на протеомика употребени во биолошките истражувања, вклучувајќи нови квантитативни и молекуларни платформи на снимање базирани на масена спектрометрија. Исто така, дадени се неколку примери од примена на овие техники во откривањето биомаркери за бубрежните заболувања.

Клучни зборови: протемика, болести на бубрезите, биомаркер, масена спектрометрија, снимање.

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