Clinical proteomics—on the long way from bench to bedside?*

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Keywords: clinical proteomics; kidney diseases; urinary proteomics

Proteomics as diagnostic tools

The increasing number of patients suffering from chronic renal failure represents one of the major challenges which the nephrologists are facing worldwide. For a better therapeutic outcome of this disease, earlier detection is urgently warranted in routine clinical practice. The standard approaches in diagnosing renal diseases remain severely limited. New techniques, such as analysis of the diseased renal proteome, are highly promising [1–6]. Beside direct analysis of renal tissue, mass spectrometric approaches to urinary peptide/protein profiling promise potential value in the non-invasive diagnosis, monitoring or prediction of renal and urinary tract diseases. First examples of such progress have been reported in a recent paper of Decramer et al. [1], who report that urinary proteomic analysis can predict the need for operation in newborns presenting with unilateral ureteropelvic junction obstruction with high significance. Furthermore, certain publications imply the possibility of an early detection of graft rejection by urinary proteomics [5,7]. Several investigators also report on specific new biomarker candidates for other renal disease detected by proteomic analysis [8–11].

What is proteomics?

Proteomics is the systematic study of proteomes, which describes the entire protein content of one or all cells of an organism as well as of bodily fluids such as blood, urine and sweat. While the genome of an organism is considered to be mostly static, the proteome shows dynamic properties with protein profiles changing depending on a variety of extra- and intracellular stimuli (i.e. cell cycle, temperature, differentiation, stress, apoptotic signals). The old paradigm ‘One gene-one protein’ is no longer valid, since many scientists have shown that the complexity of proteomes is highly influenced by the generation of protein isoforms through the mechanism of alternative splicing and post-translational modification. Today, biomedical proteome investigations are focussed on either expression proteomics, which analyses up- and down-regulation of protein levels, or on functional proteomics, aimed at identifying interacting proteins and multiprotein complexes in order to unravel molecular functions and signalling pathways of proteins. In clinical application, a comparative approach of normal and abnormal status of cells, tissues or bodily fluids is used to identify proteins that exhibit quantitative changes in a disease-specific manner, for use as diagnostic markers or therapeutic targets. Clinical proteomics is still a promising new analytic discipline with the following main aims: (i) discovery of biomarkers allowing early detection, risk management or therapeutic monitoring of diseases for the establishment of individualized treatment procedures, (ii) identification of protein targets for the development of new mechanistic intervention therapies with the promise of an improved clinical outcome. In addition, proteomics studies of bodily fluids such as urine are expected to reflect disease-related protein changes of tissue or cellular proteomes and thus provide the opportunity for the development of new non-invasive diagnostic tests and procedures, with the potential advantage of lower costs and higher efficiency of patient care. Diagnostic tools using urine are particularly promising for the early detection and

differentiation of renal deterioration before overt clinical symptoms during the various kidney-specific or associated diseases. In general, clinical proteomics has enormous potential to improve and expand non-invasive urinary protein diagnostics, particularly on the basis of a better differentiation of renal proteome pathology. Nevertheless, robustness, sensitivity, reliability and consistency of the test systems for the detection of changes in protein expression are crucial parameters, in addition to labour and cost expenses for the acceptance of proteomics studies in specific clinical settings such as renal diagnostics. At present, many proteomics techniques still suffer from insufficient standardization and only a few have the potential to fulfil essential criteria for future practical clinical application. Here we discuss the present methods used for urinary proteome analysis.

Proteomics techniques for urine proteome analysis

Gel-based proteome analysis

Two-dimensional gel electrophoresis is a powerful and widely used method for the analysis of complex protein mixtures extracted from cells, tissues or biological fluids [12]. This technique permits separation and characterization of proteins according to their charge/ion strength and molecular weight, in two consecutive gel electrophoresis steps: proteins are first separated by isoelectric focussing, according to their isoelectric points and then distinguished according to their molecular weights in SDS-polyacrylamide gel electrophoresis (Figure 1). 2-D gel-electrophoresis is generally labour- and time-intensive and without strict standardization in the applied reagents, apparatus and software for the analysis usually not routinely applicable in clinical settings.

Gel-free proteome analysis

Reduced sample requirement, high throughput and automation are also important conditions for the integration of proteomics in routine laboratories. For this reason, different methods have been developed, which effectively couple high-end mass spectrometry to array formats, to capillary electrophoresis or to chromatography. The surface-enhanced laser desorption/ionization (SELDI) technique offers such
an opportunity for urine analysis. Small amounts of native urine samples can be applied to the surface of a SELDI ProteinChip without prior concentration or precipitation of the urinary proteins [5,13]. The bound proteins may then be directly analysed by MALDI-TOF-MS (Figure 1) [14,15]. Also CE-MS coupled the high-resolution properties of capillary electrophoresis (CE) with the powerful identification ability of the electrospray time-of-flight MS to profile and sequence urinary proteins. Liquid chromatography coupled to mass spectrometry (LC-MS) also offers a gel-free alternative for sensitive urine proteome analysis. Thus, protein profiles or single identified proteins may be characterized as disease-specific protein pattern or biomarkers which, however, must be validated in controlled retro- and prospective clinical studies.

Urinary proteome analysis as clinical diagnostic tool

Different studies have already applied proteomics methods to analyse the urinary proteome and have attempted to identify markers associated with renal diseases [5,9,11,16,17]. Clinical usage of a proteomics strategy was first proven successful in the aforementioned report by Decramer et al. [1]. High-resolution properties of CE-MS were used to profile urinary polypeptides from 103 neonates suffering from ureteropelvic junction (UPJ) obstruction, a common clinical problem after birth [18]. In a first step, data from healthy newborns were compared with those of normal adults. Scattered and less reliable data were obtained from the adults compared with the newborns. In a second step, the newborns were classified at birth into three groups, depending on clinical criteria: non-operated individuals with UPJ obstruction (No_OP), individuals who might possibly undergo operation (OP_Poss) and individuals with severe UPJ obstruction who had to undergo surgery rapidly after birth (OP). The authors reported that the comparison of the polypeptides patterns allowed the identification of biomarkers discriminating between the three groups. Among the identified markers, 19 were found to differentiate between the healthy newborn No_OP group and the OP group. Furthermore, the authors detected 51 polypeptides that discriminated between the three groups. The identified polypeptides were validated in urines collected in a prospective blinded study. Using this proteomics approach and the biomarkers identified in this study the authors claim to be able to predict the clinical outcome of neonates with UPJ obstruction with 94% precision, several months in advance. This study is one of the as yet singular leading examples showing that proteomic techniques can be successfully applied to improve patients monitoring and management in a clinical setting, based on the usage of strictly standardized sampling and handling of the analytical probes, clear clinical differentiation of the patent and control group and statistical assessment of quantitative protein changes in the CE-mass spectrometrical analysis. Nevertheless, the authors must still prove, in an extended validation process, that the data hold true on larger panels of patients and controls, with a precise definition of the rate of false positive/false negative patients. In addition, like many previous proteomics analyses, the study suffers from a lack of the identification of all the relevant protein peaks at the amino acid sequence level. This would aid to further verify the value of these biomarkers and shed light on the pathophysiology of the disease. In addition, it could help to implement these biomarker panels into routine clinical diagnostics, based on the development of more cost-effective detection approaches with antibodies.

Regardless of the great promise of clinical proteomics, the identification of urinary biomarkers by spectrometry technologies for an earlier diagnosis, prognosis or prediction of therapeutic responses in renal diseases is still at the beginning. So far, many studies describing the discovery of biomarkers in urine are merely reporting on peptide patterns with lists of peptide/protein mass/charge and only in best cases show identification of a specific protein [1,5–10,19,20]. Although the methods used in these studies demonstrated their capability of high-throughput analyses and low sample load, these techniques are still limited in their detection of low abundance proteins as well as in a standardized quantification of the detected biomarkers. This includes standardized protocols of pre-analytical and analytical handling of the clinical probes such as urine, as reported (http://gwdg.de/~nephro/). Only few of these presumptive biomarkers have as yet been in larger validation studies to demonstrate their ability to specify diagnosis, prognosis or optimal treatment regimen. Most of them must still complete such processes of rigorous and sufficient validation tailored to the complexity of the respective disease in clinical trials, in order to attain broader accepted clinical usage and approval by the regulatory agencies. Thus, despite the myriads of proteins that can be identified in normal and diseased urine today with the advanced proteomics technologies, there is still a big gap between the discovery of new urinary biomarkers and their clinical utility. The reasons for that also include the strong need for inter-laboratory standardization of techniques and of interpretation of the results in the first place. These challenges can only be overcome by intensively collaborating teams of research scientists, clinicians and statisticians, also with the support of HuPO (Human Proteome Organisation http://www.hupo.org/) and HKUPP (Website of the International Human Kidney & Urine Proteome Project http://hkupp.kir.jp/), which attempt to provide organized platforms for all information available on normal and diseased human proteomes at the international level. Due to the aforementioned limitations and uncertainties, urinary proteomics at present cannot replace invasive standardized diagnostic procedures.
such as the renal biopsy, but holds great promise and potential for future highly improved diagnosis and care of the patient in nephrology.

References


Received for publication: 30.10.06
Accepted in revised form: 11.12.06