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STATE OF THE ART REVIEW

Challenges of Genomics and Proteomics in Nephrology

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An increasing number of patients suffering from renal diseases and limitations in standard diagnostic and therapeutic approaches has created an intense interest in applying genomics and proteomics in the field of nephrology. Genomics has provided a vast amount of information, linking the gene activity with disease. However, proteomic technologies allow us to understand proteins and their modifications, elucidating properties of cellular behavior that may not be reflected in analysis of gene expression. The application of these innovative approaches has recently yielded the promising new urinary biomarkers for acute kidney injury and chronic kidney disease, thus providing a better insight in renal pathophysiology and establishing the basis for new therapeutic strategies. Despite significant improvements in therapeutics, the mortality and morbidity associated with acute renal failure (ARF) remain high. The lack of early markers for ARF causes an unacceptable delay in initiating therapy. These biomarker panels will probably be useful for assessing the duration and severity of ARF, and for predicting progression and adverse clinical outcomes. Kidney failure leads to the uremic syndrome characterized by accumulation of uremic toxins, which are normally cleared by the kidneys. Proteomics has gained considerable interest in this field, as a new and promising analytical approach to identify new uremic toxins. The urinary proteome as a tool for biomarker discovery is still in its early phase. A major challenge will be the integration of proteomics with genomics data and their functional interpretation in conjunction with clinical results and epidemiology.

Keywords acute kidney injury, biomarkers, diabetic nephropathy, glomerulonephritis, genomics, proteomics, urinary proteome

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INTRODUCTION

The sequencing of the human genome was an exceptional achievement, setting the foundation for building new knowledge in medicine. Development in high-throughput measurement technologies for biological molecules has created a paradigm shift in modern research, emphasizing the holistic approach to genes and proteins, instead of traditional investigation of one gene or one protein at a time.

The increasing number of patients suffering from renal and urothelial diseases and obvious limitations in standard diagnostic and therapeutic approaches created an intense interest in applying genomics and proteomics in the field of nephrology.[1] Noninvasive diagnosis of kidney diseases and assessment of the prognosis are still challenges in clinical nephrology. Contemporary genomic and proteomic technologies offer fresh and very promising opportunities in the development of novel biomarkers for diagnosis and early detection of kidneys disease and identification of new targets for therapeutics and evaluation of therapeutic effect and toxicity. Recent advances, including gene array technology, two-dimensional gel electrophoresis (2-DE), and new mass spectrometric techniques (MS) coupled with improvements in bioinformatics tools, show great promise of meeting the demand for developing accurate simplified diagnostics and rationally designed therapies as an ultimate goal.[2]

GENOMICS

With the completion of Human Genome Project, a new era in molecular medicine began. Genomics is established as a comprehensive analysis of gene expression of a large number of genes by assessing relative or semiquantitative amounts of RNA in biological specimens, that is, the analysis of the genetic content of an organism. Genomics study the genome as a whole, and it is based

on high-throughput techniques allowing a wide picture of gene characteristics. Mutations, deletions, and epigenetic alterations that directly or indirectly alter gene expression may also be uncovered by genomic analyses.[3]

Large-scale scanning of the human genome has become possible with the introduction of the DNA microarray.[4,5] This technology is based on an orderly arrangement of a great number of specific probes in a reduced space, allowing analysis of the whole genome on a single chip. The ability to survey the expression of 5,000 to 50,000 genes in a single experiment provides valuable new opportunities as well as new challenges. The use of DNA microarray to detect global gene expression changes for clinical purposes is now widely used in complex disease research.

Nearly all of the genes indicated for human hereditary monogenic diseases have been identified. The greater challenge will be to unravel the polygenic disease that is under the influence of multiple genes. Now that the whole genome is sequenced, there are ongoing efforts to identify genetic polymorphisms (e.g., single nucleotide polymorphisms, SNPs) that may point to disease predisposition, or unique response to therapy such as drug adverse effects.[6] It is now possible to interrogate more than 1.5 million SNPs distributed over the entire human genome. SNPs are viewed as ideal markers for large-scale genome-wide association studies to discover genes in a number of common complex diseases that are prevalent in nephropathology.

Genomic Approach in Nephrology

The kidney is characterized by a high level of cellular heterogeneity. The study of gene expression in dissected renal lobes of adult human kidneys using DNA microarrays revealed the unique and highly distinctive patterns of gene expression for glomeruli, cortex, medulla, papillary tips, and pelvic samples. Immunohistochemical staining using selected antisera confirmed differential expression of several cognate proteins and provided histological localization of expression within the nephron.[7]

Genes for the inherited renal diseases have been found, and type of inheritance is defined. One of the well-known inherited diseases of the kidney is polycystic renal disease. It can be inherited as an autosomal dominant trait (ADPKD) and autosomal recessive trait (ARPKD). ADPKD is genetically heterogeneous and can arise from mutations in two genes, namely, PKD1 and PKD2. All typical cases of ARPKD are due to mutations of the PKHD1 gene on chromosome 6.[8] Familial juvenile nephronophthisis (NPH), an autosomal recessive cystic disease of the kidney, is the most common genetic cause of end stage renal disease in the first two decades of life. A gene locus for nephronophthisis type I has been mapped by linkage analysis to chromosome 2q13.[9]

Analyzing genetic polymorphisms recently became a cornerstone in nephrology investigations. Immunoglobulin A nephropathy (IgAN) is the most common type of primary glomerulonephritis in the world among patients undergoing renal biopsy and an important cause of end stage renal disease. Vuong et al. analyzed several SNPs in a region of transforming growth factor-β1 (TGFβ1) gene, known to be a contributor to the proliferation and development of fibrosis in renal tissue, in biopsy-proven IgA nephropathy patients. Their experimental data together with the meta-analysis suggest TGFB1 as an important candidate gene for further biological studies of IgA nephropathy and as a possible target for therapy.[10] Genomic analysis also showed the relationship between the progression of IgAN and the A1818T polymorphism in intron 2 of angiotensin II type 2 receptor (AT2R) gene, which might play protective roles in the pathogenesis of IgAN.[11] A recent study suggested that the heme oxygenase-1 gene promoter length polymorphism was related to the renal impairment of IgA nephropathy at diagnosis, which is an important risk factor for mortality in IgA nephropathy patients.[12]

Genetic alterations are demonstrated in some other forms of glomerulonephritis. Mutations of two proteins exclusively expressed by the podocytes, podocin and alpha-actinin-4, in familial forms of focal segmental glomerulosclerosis are discovered. Mutations of gene for podocin (NPHS2 gene) are responsible for the autosomal recessive form of steroid-resistant nephrotic syndrome. These observations support a role of molecular screening of the podocin gene in patients with nephrotic syndrome before immunosuppressive treatment is started.[13]

Despite extensive evidence for genetic susceptibility to diabetic nephropathy (DN), the identification of susceptibility genes and their variants has had limited success. Recently, a genome-wide association scan was performed for DN susceptibility genes in type 1 diabetes mellitus. A total of 13 SNPs located in four genomic loci were associated with DN, with the strongest association at the FRMD3 and CARS loci.[14]

Acute kidney injury (AKI) is a major clinical problem with a rising incidence and high mortality rate. The application of functional genomics to human and animal models of AKI has uncovered several novel genes that are emerging as biomarkers and novel therapeutic targets. Detailed mouse kidney microarray analyses at early time points after ischemia-reperfusion injury were performed to identify consistent patterns of altered gene expression, including transcription factors, growth and regenerative genes, and apoptotic molecules.[15] The results pointed out FADD, DAXX, BAD, BAK, and p53, all of which were

confirmed by immunohistochemistry, indicating that apoptosis is a major mechanism of early tubule cell death in contemporary clinical acute renal failure (ARF). Consequent proteomic studies identified a multitude of apoptotic pathways that are activated in tubule cells following human ARF, and the inhibition of apoptosis has emerged as a promising approach in human ARF.[16]

Genomic approaches have revolutionized the field of cancer research. In a comparison of most common pathohistological types of renal tumors to normal kidney, a common set of 31 genes that were overexpressed in renal cell cancer, transitional cell cancer, and oncocytomas was discovered. This 31-gene list includes several genes that play a critical role in cancer, such as transforming growth factor-β2 (TGF-β2), a disintegrin and metalloproteinase 12, and protein kinase C, indicating that some common biological mechanisms may be involved in most renal tumors.[17]

Renal cell cancer (RCC) is the most lethal of the urological cancers and accounts for 3% of all adult malignancies. Commonly reported genes that are highly expressed in the different subtypes of RCC are as follows: VEGF, endothelin 1, solute carrier family 2, insulin-like growth factor–binding protein 3 in clear cell RCC, a-methyl-CoA racemase in papillary RCC, and KIT (CD117) in chromophobe RCC.[17] Genomic analysis of RCC accumulated ample data that now can be exploited in clinical management of an almost uncontrollable disease. In addition to the previously identified genetic abnormalities (i.e., VHL, MET, EGFR), CAIX seems to be a novel molecular marker of RCC. Array studies also outlined a small set of tumor markers—namely, vimentin, galectin-3, CD74 and parvalbumin—which can define the individual histologic subtypes of RCC. Further novel molecular targets are available, such as HIF, HSP90, or the IFN-regulated genes, which can be used to the fine-tuning of RCC therapy.[18]

PROTEOMICS

Proteomics is "the systematic analysis of proteins for their identity, quantity, and function."[19] It is the systematic study of a proteome, 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.[1]

Genomics has provided a vast amount of information, linking gene activity with disease. However, genomic studies give no indication of the complexity of protein–protein interactions, posttranslational modifications, and state of the cellular circuitry. The dynamic nature of the proteome imposes the reason for investigating gene expression in diseases directly at the proteomic level. As proteins are the ultimate effectors molecules, proteomics is the ideal complement of genomic approach.

Proteomics technologies allow us to understand proteins and their modifications, which may elucidate properties of cellular behavior that may not be reflected in an analysis of gene expression. Proteomics development can be attributed primarily to the refinements in mass spectrometry, improvements in computer and software sciences, and the flood of data now available from genomic sequencing.[20]

Clinical proteomics is an exciting subdiscipline of proteomics that involves the application of proteomic technologies at the bedside, bridging the capability and the utility of proteomics in nephrology and medicine.

Proteomic Approach in Nephrology

Most of the kidney diseases leading to end stage renal disease are characterized by silent and progressive course with few, if any, nonspecific symptoms and signs. Noninvasive diagnosis and assessment of the prognosis are still challenges in clinical nephrology. Proteomics is expected to provide a solution by defining specific disease biomarkers, which will allow an early diagnosis, progression, and therapy response monitoring.

During the past decade, proteomics has been extensively applied to nephrology and has become a very fruitful field.[21,22] Several renal centers have initiated proteomic analysis to their research areas. Promising areas of research include a description of altered protein expression in tissues and biological fluids (serum and urine), the development of novel biomarkers for diagnosis and early detection of kidneys undergoing end-stage renal disease, the identification of new targets for therapeutics, and the potential for accelerating drug development through more effective strategies to evaluate therapeutic effect and toxicity.[23]

Proteomics requires in its different stages of realization various technological platforms with high sensitivity and high throughput. Because of the inherent complexity of a proteome, all approaches for its examination generally rely on a separation step, using either gel-based (2-dimensional electrophoresis (2-DE), differential in-gel electrophoresis (DIGE)), or gel-free method (array formats, liquid chromatography, capillary electrophoresis), followed by ionization and subsequent mass spectrometry analysis (surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) or matrix assisted (MALDI-TOF MS).

At present, many proteomic techniques still suffer from insufficient standardization, and only a few have the potential to fulfill essential criteria for future practical clinical application. There is a strong need for inter-laboratory standardization of the techniques and of the interpretation of the results at the first place.[20,23] Further technological innovations would be beneficial to increase sensitivity,

reduce sample requirement, increase throughput, and more effectively uncover various types of protein alterations such as post-translational modifications.

Proteomics of the Kidney

The analysis of kidney's proteome encompasses several disadvantages: the kidney is composed of different cell types, all of which express different and specialized proteomes, and tissue samples must be obtained invasively. That imposes ethical issues and is a major obstacle in acquiring normal kidney tissue as a control sample.[24] Therefore, most research has focused on tissue that is obtained from experimental animals.

The proteome of the rat kidney was described,[25] showing the differential expression of proteins in the renal cortex and medulla. 2-DE resolved 1095 spots from the cortex and 885 spots from the medulla. MALDI-TOF MS analysis identified 54 unique proteins, nine of which were differentially expressed in the cortex and medulla. An examination performed on glomeruli obtained by laser capture dissection from a tissue in the five-sixths nephrectomy rat model of FSGS on a subsequent proteome analysis showed that closely related proteomic patterns of nonsclerotic and sclerotic glomeruli suggest early activation of presclerotic mechanisms even in seemingly intact glomeruli, identifying Thymosin β4 as a marker of such early events that may even contribute to sclerosis.[26] Using combined proteomic and transcriptomic profiling, researchers also established such protein map from murine tissue.[27]

Limitations of data obtained from proteomic analysis of the whole kidney are that major abundant proteins may obscure the identification of lower abundant proteins and do not provide any information regarding localization. In an attempt to construct a proteome map for normal human glomeruli from approximately 1,600 visualized protein spots, more than 100 proteins were identified by MALDI-TOF MS and nanoelectrospray ionization MS.[28] These studies that attempted to explore individual proteomes of intrarenal structures will be very useful and provide lots of information for future physiological study.

Urinary Proteomics

Proteome research related to nephrology has generally focused on the examination of urine because it is easily accessible in a large quantity. Urine is a messenger of the urinary system function and disorders; thus, it is considered that urinary diagnostics can help to detect diseases that do not produce striking signs or symptoms at an early stage. Proteomics has enormous potential to improve the quality of urine protein-based diagnostics, as well as providing practical insights that will impact medical practice and therapy.[23,29]

Noninvasive accessibility of urine makes it attractive for proteomic research. It is found that midstream sample of the second morning urine is optimal and was already used in several studies.[30,31] Urinary proteins have been shown to remain stable long enough to perform reliable proteome analysis. In addition, urine can be stored for several years, even at -20°C, without significant alterations in its proteome.[24] The main challenges in working with urine for biomarker discovery are in the standardization of urine sample collection, storage, shipment, enrichment of potential markers with low abundances, and quantification of the excretion rate from a single marker. The large variation in protein concentration in urine from different individuals is also a big challenge for disease biomarker discovery and quantitative proteomics.

Protein Expression Profiles in the Normal Urine

The first human urinary proteome map, consisting of 67 proteins and their isoforms, that could be used as a reference was defined by Thongboonkerd et al. (2002) using acetone-precipitated urine samples from healthy volunteers.[32] Additional experiments that further expanded the knowledge of the normal urinary proteome were reported by Pieper et al. (2004), who identified 150 unique proteins [33]; Sun et al. (2005), who identified 226[34]; and Castagna et al. (2004), who reported identification of 295 unique proteins from the exosome.[35] Taken together, these approaches have identified approximately 800 proteins and laid the foundation for the subsequent discovery of biomarkers in the urinary proteome. Adachi et al. (2006) identified more than 1500 proteins and peptides in the urine of healthy individuals, many of which were represented by membrane proteins, probably due to the presence of exosomes.[36]

Urinary Proteomics: A Tool to Discover Biomarkers for Kidney Disease

The main focus of proteome analysis in nephrology is on detection and identification of urinary proteins that significantly change (in abundance, distribution, etc.) during pathophysiologic changes of the kidney structure and/or function.[24] Some of detected proteome changes in specific states may potentially be new therapeutic targets or novel biomarkers for disease detection or prognosis. Biomarkers represent a catalytic event in the interplay between academia and industry, offering the opportunity to have an impact on patient health in a more economical manner by earlier disease detection and the possibility to predict which patients will respond to which therapies.[37]

Despite significant improvements in therapeutics, the mortality and morbidity associated with ARF remain high. The lack of early markers for ARF causes an unacceptable delay in initiating therapy. Exosomal fetuin-A was proposed as biomarker of acute kidney injury, based on data from a rat model, which were further supported by Western blots on patients.[38] Recent advances in proteomics that hold promise in ischemic ARF include a plasma panel [neutrophil gelatinase-associated lipocalin (NGAL) and cystatin C] and a urine panel [NGAL, interleukin-18, kidney injury molecule-1 (KIM-1), cystatin C, α1-microglobulin, fetuin-A, Gro-alpha, and meprin]. These biomarker panels will probably be useful for assessing the duration and severity of ARF, and for predicting progression and adverse clinical outcomes. It is also likely that the biomarker panels will help to distinguish between the various etiologies of ARF. It will be important in future studies to validate the sensitivity and specificity of these biomarker panels in clinical samples from large cohorts and in multiple clinical situations.[39]

The detection of biomarkers for various glomerular diseases is one of the most useful applications of proteomics to nephrology. In proteomic analysis of urine from patients with IgA nephropathy, the authors found an array of differentially present proteins and used the data to initiate the establishment of a human urinary proteome map of IgA nephropathy.[40] In a recent study of focal segmental glomerulosclerosis (FSGS), 37 urinary proteins were identified, showing characteristic patterns of dynamic changes along the disease course of FSGS.[41] Some urinary proteins appearing earlier than glomerular sclerosis could serve as potential early diagnostic biomarkers. The proteins with the pathogenic roles could serve as potential non-invasive prognostic markers of FSGS, and give insight into the pathogenic mechanisms of this sclerosis disease.

Specific urinary proteome analysis in patients with type 2 diabetes has shown that a total of 168 urinary proteins were present in more than 90% of the samples, suggesting the existence of a consistent urinary proteome. Panels of protein markers allowed not only the diagnosis of a specific primary kidney disease but also the discrimination with high sensitivity and specificity between different kidney diseases, such as IgA nephropathy, FSGS, membranous glomerulonephritis, minimal-change disease, and diabetic nephropathy.[42,43]

Diabetic nephropathy (DN) is the leading cause of end stage renal disease, and is constantly increasing, due to the rising prevalence of diabetes mellitus type 2. Alpha1-Antitrypsin was identified as a potentially upregulated biomarker.[44] Recent data suggest that one processed form of ubiquitin UbA52, fusion protein located in renal tubules, was missed in the urine of diabetic nephropathy patients. This ubiquitin form could be used as a prognostic marker for DN.[45] The ubiquitin degradation assays confirmed the potential role of a urinary protease, the absence of which was specific for diabetic nephropathy. The identification of this protease will determine the usefulness of the short form of ubiquitin as a marker for predicting the clinical course and the potential role of the protease in the pathophysiology of diabetic renal involvement.

In an attempt to detect potential biomarkers for allograft rejection in kidney transplant patients, urinary proteomic analysis revealed several proteins that substantially differentiated in concentration in the urine of patients who received a transplant and healthy individuals. Moreover, several potential biomarkers for acute rejection could be defined.[46]

Urinary proteome analysis may also be an excellent tool for fast, noninvasive, and unbiased monitoring of disease progression or response to therapy. In a study that evaluated one angiotensin II receptor blockage drug efficiency in patients with microalbuminuria, the treatment resulted in a significant change in 15 of 113 proteins that are characteristic for diabetic renal damage.[47]

Despite the enthusiasm and interest in this field, the urinary proteome as a tool for biomarker discovery is still in its early phase. The majority of studies were performed with a small number of individuals. From the large number of detected potential marker candidates, only a few will fulfill the criteria required for a good biomarker. Furthermore, for the diagnosis of complex diseases like renal diseases, only a multimarker assay could deliver a better diagnosis and allow therapeutic strategies that delay or prevent the prognosis of the disease.

Kidney failure leads to the uremic syndrome that is the clinical expression of the malfunction of vital organs due to the accumulation of uremic toxins, which are normally cleared by the kidneys. Proteomics has gained considerable interest in this field as a new and promising analytical approach to identifying new uremic toxins. For polypeptides >10 kDa, classical proteomic techniques, such as two-dimensional gel electrophoresis followed by mass spectrometry, are able to identify uremic polypeptides. In the mass range from approximately 1 to 10 kDa, capillary electrophoresis coupled to mass spectrometry (CE-MS) emerged as a possibility to quickly analyze up to 1,400 compounds in a single step.[48]

Great efforts are currently focused on characterization and staging of various neoplasms, including renal cell carcinomas and urothelial tumors. Proteomic methods have also been applied to the investigation of urological malignancies. Renal cell carcinoma (RCC) represents one of the major health problems because of its morbidity and mortality rate. As early symptoms are rare and nonspecific, more than 30% of patients already have metastases at the

time of diagnosis and poor prognosis, with nephrectomy remaining the most frequent treatment. Currently, no single protein marker has been proven to be useful for either detecting or monitoring RCC development and treatment. Proteomic approaches in the form of comparative 2-DE analysis of normal renal and RCC tissues revealed a number of proteins associated with RCC. Ubiquinol cytochrome c reductase, NADH-ubiquinone oxido-reductase complex I, and two isoforms of plasma glutathione peroxidase were down-regulated in RCC. It was demonstrated that renal tissue in RCC patients expressed two multimeric and five monomeric forms of Mn-superoxide dismutase (SOD), whereas normal kidney expressed only two monomeric Mn-SOD spots without multimeric form.[49]

Most bladder cancers are transitional cell carcinomas (TCC) that arise from the epithelium lining the urinary drainage system. Squamous cell carcinoma is much less common. Celis et al.[50] have performed thorough 2-D gel analysis of tumor tissue from both types of bladder cancer to create a reference database useful in biomarker discovery endeavors. In experimentally induced TCC in rats, two proteins emerged as potentially valuable markers: cytokeratin-20 (CK-20), which was over-expressed, and seminal vesicle secretary protein VI (SVS-VI), which was under-expressed in hyperplastic tissues.[51] The inflammation-associated calcium binding protein S100A8 (MRP-8, calgranulin A) is reported to be highly expressed in tumor cells in contrast to normal urothelium.[52] These new markers, when fully characterized, may contribute to new target proteins for the prediction of aggressive, invasive bladder tumors.

In the urine of patients with bladder squamous cell carcinoma (SCC), 124 polypeptides were identified, and among them only psoriasin was expressed exclusively in the urine from SCC patients.[53] This was consistent with the knowledge that psoriasin is a major abundant protein in human keratinocytes and would be expected to be present in the SCC urine. Psoriasin alone, or in combination with other markers, is therefore a promising biomarker to detect bladder SCC.

To date, there is no validated and unique biomarker for the detection and monitoring of urological cancer evolution and treatment. The eventual urinary test may consist of multiple assays detecting nucleic acids as well as proteins. In addition, the test should also be able to reveal to the clinician both prognostic information and therapeutic targets for personalized medical treatment.

CONCLUSION

It is clear that genomics and proteomics represent powerful and promising tools in current nephrology research. The difficult question is how to translate the provided vast amounts of data into a meaningful clinical context, and how to translate this information into clinical trial design and subsequently into routine clinical use. The findings in the rapidly evolving field of genomics and proteomics may ultimately complement histopathological analysis, the current diagnostic and prognostic gold standard.

Proteomics offer a new technology platform for the identification and quantification of novel urinary biomarkers that may lead to the development of simple, noninvasive, safe, and accurate tests to be used in clinical practice for earlier disease detection and better therapeutic outcome. Even if the implementation of full proteomics studies into clinical laboratories is beyond the scope of current science, proteomics is already delivering tangible benefits, deciphering the molecular basis of renal diseases and identifying new diagnostic and therapeutic targets. A major challenge will be the integration of proteomics with genomics data and their functional interpretation in conjunction with clinical results and epidemiology. This will allow us to obtain the holistic molecular view of pathogenetic processes, which enables their early recognition and the best selection of therapy tailored to the individual patient.

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