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Abstract

With the advent of transcriptome profiling techniques, an enormous amount of data has been generated in the field of molecular nephrology. We will review analysis tools and challenges for genomic approaches and present their application in gene-expression studies on kidney biopsies. The findings in this rapidly evolving field may ultimately complement histopathological analysis, the current diagnostic and prognostic gold standard. Altogether, genomics may bring nephrology one-step closer to a systematic understanding of biological processes involved in renal disease.

Genomic Analysis in Nephrology – towards Systems Biology and Systematic Medicine?

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Abstract

English:

With the advent of transcriptome profiling techniques an enormous amount of data has been generated in the field of molecular nephrology. We will review analysis tools and challenges for genomic approaches and present their application in gene expression studies on kidney biopsies. The findings in this rapidly evolving field may ultimately complement histopathological analysis, the current diagnostic and prognostic gold standard. Altogether genomics may bring nephrology one step closer to a systematic understanding of biological processes involved in renal disease.

French:

Depuis l'introduction des techniques pour l'étude du profil du transcriptome beaucoup de données ont été créées dans ce domaine de la néphrologie moléculaire. Nous allons présenter et résumer les méthodes d'approches génomiques (par exemple puces ADN et bioinformatique) et les études récentes sur les biopsies rénales. Les résultats obtenus par les expériences dans ce secteur scientifique en pleine évolution pourraient compléter l'analyse classique histopathologique, comme source supplémentaire pour les diagnostiques moléculaires fondés sur les méthodes conventionnelles.

En résumé, la génomatique permettrait aux néphrologues d'aller plus loin dans la compréhension systématique des processus biologiques.

1. Introduction

In nephrology therapeutic decisions are often based on histopathological results of renal biopsies. But kidney biopsies still offer limited predictive strength and therapeutic consequence, e.g. in the nephrotic syndrome [1]. As correlation of histopathologic features with kidney function, e.g. creatinine clearance and outcome, is not always sufficient [2], additional sources for diagnostic information are desirable. In haematology and oncology gene expression analysis has added important information to routine diagnostic strategies. This has led to the implementation of gene expression in the state-of-the-art workup of specific diagnostic samples [3].

With sequencing of the human genome intriguing opportunities in clinical and experimental nephrology arise. It is now possible to analyse the transcriptome, i.e. tens of thousands of mRNAs expressed in a given tissue at a specific time. These genomic data have led together with similar –omic approaches (e.g. proteomics) to the development of systems biology, which aims to decipher on a global level the biological networks activated in health and disease.

Gene expression analysis on renal tissue of diseased humans may allow the nephrology community to address two main topics:

1. “How can we increase the impact of information we get from a renal biopsy (e.g. disease categories, response to therapy, patient outcome)?”
2. “What are interconnected biological pathways activated in the respective clinical setting?”

Altogether this may lead to more specific diagnoses, individualised therapies and a better understanding of renal disease (see figure 1). In this review we want to present selected gene expression studies performed on human renal tissue. All of these studies have laid the path to the above aims.

2. Tools in genomic nephrology

Three methods used in modern gene expression profiling deserve special consideration: Real-time reverse transcription polymerase chain reaction (rt-RT-PCR), microarrays and serial analysis of gene expression (SAGE).

2.1. RT-PCR

Real time-RT-PCR is an ultra-sensitive technique, which allows to rapidly quantify minute amounts of target mRNAs by determining the number of amplicons after each PCR cycle. With this approach it is possible to quantify mRNA expression from single glomeruli and even single cells, e.g. podocytes [4].

Expression levels of the molecule of interest have to be related to the amount of tissue analysed. This obstacle can be overcome by using several reference RNAs in parallel. These should have stable expression levels and are referred to as reference genes or housekeepers [5].

2.2. Microarrays

While a major shortcoming of RT-PCR studies is the limited number of genes that can be examined, microarrays allow us to study the expression of several thousands of genes in one reaction. A microarray is a matrix of thousands of molecules (for gene expression studies oligonucleotides or cDNA probes) imprinted on a solid support. Labelled mRNA/cDNA from the tissue of interest hybridises to its complement sequence on the array. Measuring signal intensity allows quantification of mRNA abundance. The hallmark of the microarray experiment is the expression profile, i.e. the sample's pattern of gene expression. A major limitation is the requirement of significant amounts of high quality mRNA as starting material. mRNA amplification before microarray analysis is an option but concerns have been raised about amplification bias.

Microarrays have proven to be an excellent vehicle to gather expression data and create hypotheses [4, 6, 7].

2.3. Serial Analysis of Gene Expression (SAGE)

A third approach, SAGE (serial analysis of gene expression), was developed based on the experience from large-scale sequencing projects. In SAGE base pair sequence tags corresponding to unique mRNAs are sequenced in a tandem-ligated form. In a second step they are compared with a genomic database. Using SAGE thousands of mRNA SAGE tags can be studied. Thus, like microarrays, SAGE can provide a quantitative profile of expressed genes [8].

3. Challenges

A number of obstacles have to be overcome for gene expression analysis of renal diseases:

3.1. Addressing the complex architecture of the kidney

Unlike the monoclonal neoplastic tissue used in the pioneering studies in haematology, the kidney is characterised by a high level of cellular heterogeneity.

Three genomic studies on physiologic human kidneys revealed a high degree of compartmentalisation. Each compartment showed unique, reproducible and highly distinctive expression patterns. These genetic patterns correlate very well with different physiologic functions attributed to a given part of the kidney [9, 10]. Using SAGE Chabardès-Garonne *et al.* were able to study nephron-segment-specific gene expression patterns [11] and Takemoto *et al.* established a comprehensive glomerular expression profile of the mouse by advanced molecular and bioinformatic techniques [12].

Thus, when studying a given condition it is preferable to focus on a certain compartment rather than using whole renal lysates. This can be achieved by manual or laser microdissection techniques [13, 14]. It should also be noted that after screening for changes in mRNA expression these can be further localised to specific glomerular or tubulo-interstitial renal cells or even cell compartments by using conventional methods, e.g. immunohistochemistry or cell culture experiments. Such approaches may be completed in the future by advanced imaging methods, e. g. intravital 2-photon microscopy [15].

3.2. Focal nature of kidney disease

Along with the limited amount of tissue obtained by a fine needle biopsy, patchy patterns of potentially multi-staged lesions may result in considerable sample bias [4].

3.3. Clinical and molecular heterogeneity

In nephrology the clinician faces a significant heterogeneity in confounding factors (e.g. diabetes, hypertension) and in the renal disease *per se* (subgroups of the same disease, e.g. WHO stages I to V in Lupus Nephritis). Thus, it is a challenge to create statistically sufficient patient cohorts for a reliable and valid study even of a common disease.

To further complicate matters there may be pathogenetic heterogeneity within a disease entity and different diseases can show similar histopathologies (e.g. glomerulosclerosis). One of the best approaches to overcome the clinical and molecular heterogeneity is to create large enough patient cohorts for statistically feasible subgroup analysis.

3.4. Bioinformatics

In order to deal with the vast and often puzzling datasets obtained in microarray experiments a thorough bioinformatic workup is crucial. Using the software tools of cluster analysis, so called dendrograms can be created whose tree structure hierarchically visualises groups of samples with similar expression patterns [16]. Computer-aided interconnection of expression profiles with libraries of biological processes and molecular functions guides towards the application of systems biology, integrating data sets into their functional context.

3.5. From genes to proteins

It is important to realise that besides mRNA abundance there are other ways to influence biological processes: splicing of RNA, posttranslational modification of proteins and protein interaction. Thus, expression results on gene level have to be confirmed on protein level and if possible linked with gene function and activity. In complex human tissues available means are limited and immunohistochemistry is the most widely used approach.

4. Multi-centre renal gene expression study in Europe

The logistic and biological issues mentioned above can best be addressed in a multi-centre setting. To this end the European Renal cDNA Bank – Kroener-Fresenius Biopsy Bank (ERCB-KFB) was initiated in 1998. More than 20 European nephrology departments and nephro-pathologists have joined in a cooperation to study gene expression and follow a protocol for high throughput application of gene expression analysis of renal biopsies: After informed consent a minor part of a routine biopsy core is transferred to a pre-coded vial filled with RNase inhibitor (RNA later, Ambion). The biopsy specimens are shipped on cool packs together with anonymous clinical data sheets and histology reports to the core facility. Here glomeruli and tubulo-interstitium are manually microdissected and mRNA is isolated from these dissected compartments. Gene expression analysis is performed by both real-time RT-PCR and microarrays (Fig. 2) [13]. A second protocol for RT-PCR analyses on formaldehyde fixed-paraffin embedded archival tissue made retrospective molecular studies on routine renal biopsies possible [14].

5. mRNA analysis of candidate genes in native renal biopsies

In the following we will give some examples of successful gene expression studies in nephrology. This summary is by far not complete and very interesting studies remain unmentioned.

5.1. Glomerulosclerosis- a final common pathway

Many different renal diseases eventually lead to the same histopathologic pattern: glomerulosclerosis. In a pioneering study on renal gene expression Esposito *et al.* [17] shed light on the dysregulated composition of the underlying collagen synthesis.

5.2. Building bridges between genes and clinical data

In a proof of principle study on hydronephrotic kidneys Henger *et al.* [18] categorised renal lesions using a molecular, gene expression-based approach. They found a correlation between gene expression fingerprint and prognosis. In the field of translational research Schmid *et al.* [19] were able to distinguish focal segmental glomerulosclerosis and minimal change disease by glomerular gene expression.

5.3. Membranous Glomerulonephropathy (MGN)

Formation of subepithelial immune complexes in MGN results in changes in the glomerular filtration barrier. In an „oeuvre-clé“ in molecular nephrology neutral endopeptidase (NEP) was identified as the first known human glomerular antigen in a specific clinical subgroup of MGN [20, 21]. Cohen *et al.* [22] found a significantly higher CD20 mRNA expression in MGN, which points towards a role of immigrating B cells in this disease. Thus, B cell targeted therapies, e.g. Rituximab, may be employed in a selective manner [23].

5.4. Lupus Nephritis (LN)

LN has been extensively studied in animal models. Fewer studies have evaluated molecular mechanisms in human LN. Using microarray technology on human glomeruli Peterson *et al.* [24] demonstrated that B cells, different myelomonocytic lineages, expression of type I interferon inducible genes and cell proliferation are involved in LN.

5.5. Rapidly Progressive Glomerulonephritis (RPGN)

RPGN can be caused by completely different and incompletely understood mechanisms. Recently Ding *et al.* [25] could identify the loss of von Hippel-Lindau antigen (VHL) as one potential mechanism, which leads to up-regulation of hypoxia-related genes. Blocking one of these genes, chemokine receptor CXCR4, proved to be an attractive therapeutic option in a rodent model.

5.6. Diabetic Nephropathy (DN)

DN represents the leading cause of end-stage renal disease in the industrialised world. Paradoxically, little is known about its molecular mechanisms.

Baelde *et al.* [26] and Lindenmeyer *et al.* [27] could show by two independent expression studies that unlike in animal models vascular endothelial growth factor A (VEGF-A) is down-regulated in human DN. This may contribute to vascular rarefaction, tubulointerstitial fibrosis and thus progression towards end-stage renal disease.

6. Molecular approaches to renal transplant recipients

6.1. Post-ischaemic renal failure

A significant number of cadaveric renal transplant recipients develop post-ischaemic acute renal failure, which is known to influence long-term graft outcome. Using a microarray-based approach on cadaveric donor kidneys Hauser *et al.* [28] and Kainz *et al.* [29] identified an up-regulation of proinflammatory pathways as potential predictors of post-ischaemic renal failure and one-year allograft function, respectively.

6.2. Chronic transplant failure

While many acute immune-mediated rejection episodes can be treated effectively, chronic allograft nephropathy still represents a major challenge in renal transplantation.

Correlating expression levels of genes significantly regulated in acute rejection with clinical follow up, Eikmans *et al.* [30, 31] found that up-regulation of certain genes (e.g. S100A8/9, TGFbeta) may predict a lower risk of developing chronic graft failure. High expression of Surfactant Protein-C, a protein originally known to be involved in alveolar stability [30], and high expression of B cell clusters, e.g. CD20 [32], may predict a rather unfavourable course.

6.3. Addressing calcineurin inhibitor associated toxicity

It is crucial but difficult in the current clinical setting to differentiate between chronic graft dysfunction and toxicity of calcineurin inhibitors. Studying expression levels of candidate genes, e.g. TGFbeta and laminin, may provide additional molecular information to guide clinical therapy [33, 34].

All the studies listed above show promising results and most have validated their results by additional means such as RT-PCR or immunohistology. However, reliabilty and validity of positive findings are further increased if confirmational data from independent patient cohorts are available. Therefore large-scale biobanks will be important not only for initial analyses but also for confirmatory studies.

7. Towards Systems biology

Systems biology builds networks of interacting biological elements, e.g. genes, to help understand the function of the whole organism, using information from a wide variety of disciplines. Thus, systems biology has been described as the search for the syntax of biological information [35]. Three studies tried to integrate such syntactic approaches to decipher biological processes involved in renal disease. Computer-based gene promoter analysis helped in all three studies to better understand renal gene expression data. Kainz *et al.* [36] used for the first time computer-based promoter analysis to search for common promoter characteristics for co-regulated genes. They found similarities in transcription factor binding site motifs in genes commonly regulated in transplant organs. Schmid, Boucherot, Yasuda *et al.* [37] could show that a master inflammatory pathway is activated in progressive diabetic nephropathy. By analysing array data from DN the induction of NFkappa B (NFkB) target genes was over-represented. Futher computer-assisted promoter analysis deciphered the specific relevance of the transcription factor binding sites for NFkB and interferon regulatory factor in progressive disease. Cohen *et al.* [38] managed to characterise shared promoter structures of molecules linked to the unique functional unit of the glomerular slit diaphragm by applying state-of-the-art promoter modelling tools. Cadherin 5, a gene previously unrecognised in this context, along with nephrin and ZO-1 were found to share specific transcription factor binding sites in their respective promoter region. All three genes were also found to be co-expressed and -regulated in different human glomerular diseases.

Of note, the chances and opportunities brought about by systems biology extend far beyond promoter analysis. The multidimensional and comprehensive approach of systems biology, including also proteomics, computer science, mathematical modelling and others, will crucially contribute to the success of many future projects in molecular renal research.

8. Conclusion: on the eve of the “âge d’or” in nephrology?

In this review, we could point out some of the many promising developments and exciting options, which are opening new avenues towards a systematic understanding of renal disease. This more comprehensive approach to renal disease may help us to establish a more systematic, mechanism-based medicine with individually tailoured therapies. And it may also help to better understand the biological systems involved in renal disease.

Focusing the power of molecular analysis on the kidney has provided a few answers– and given raise to many new questions.

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Figure legends:

Figure 1: Schematic overview of genomic approaches in medicine

Clinical data (e.g. histological diagnosis, specific lab values) are correlated with genomic data (e.g. microarray). Regulation of specific genes is confirmed by other means (RT-PCR, immunohistochemistry). To understand the role of the regulated genes further experiments are needed *in vitro* and *in vivo*. At the end, a more comprehensive understanding of the biological processes can be achieved (here symbolized by a network). This may lead to the development of modern therapies or to the generation of more specific diagnosis criteria.

Figure 2: The European Renal cDNA Bank – Kroener-Fresenius Biopsy Bank

A small part of a renal biopsy is transferred to RNase inhibitor. Glomeruli and tubulo-interstitium are manually microdissected at the core facility of the study. Expression analysis can be performed by real-time RT-PCR or microarrays. These data can be correlated with histology and clinical data.

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