The Promise of Systems Biology for Diabetic Kidney Disease

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Diabetic kidney disease (DKD) has a complex and prolonged pathogenesis involving many cell types in the kidney as well as extrarenal factors. It is clinically silent for many years after the onset of diabetes and usually progresses over decades. Given this complexity, a comprehensive and unbiased molecular approach is best suited to help identify the most critical mechanisms responsible for progression of DKD and those most suited for targeted intervention. Systems biological investigations provide such an approach since they examine the entire network of molecular changes that occur in a disease process in a comprehensive way instead of focusing on a single abnormal molecule or pathway. Systems biological studies can also start with analysis of the disease in humans, not in animal or cell culture models that often poorly reproduce the changes in human DKD. Indeed, in the last decade, systems biological approaches have led to the identification of critical molecular abnormalities in DKD and have directly led to development of new biomarkers and potential treatments for DKD.

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Diabetic kidney disease (DKD) is the major cause of CKD and ESRD in the United States and probably in the world.[1] Despite some progress in reducing mortality and delaying kidney disease in the last few decades due to improved glycemic control, blood pressure lowering, and the use of renin-angiotensin system blockade, the percentage of diabetic patients who develop kidney failure has not materially declined.[2] Thus, millions of individual patients worldwide are in urgent need of new approaches to treatment that will actually prevent progression to ESRD. One reason for the slow progress in finding adequate therapies for DKD is the lack of comprehensive understanding of the underlying pathogenic mechanisms. Unfortunately, targeting single pathways and molecules based on hypothesis-driven research has resulted in no significant advances in treatment in the last 25 years. Unraveling the underlying mechanisms for DKD is complicated by the likelihood that a number of molecular processes interrelate over a number of years to cause the tissue damage that is the ultimate manifestation of the disease. A comprehensive and unbiased molecular approach is best suited to reveal this complex pathogenesis. Systems biological methods provide such an approach and have the additional important advantage that they can start with human disease samples to help ensure that the processes being studied are relevant to human DKD. This is important since animal and cell culture models fail to recapitulate many aspects of DKD found in humans.[3] In this brief review, we will discuss how a systems biological approach to understand and treat DKD has advanced the field over the last decade or so.

SYSTEMS BIOLOGICAL APPROACHES TO DIABETIC KIDNEY DISEASE

There are many definitions to Systems Biology and different definitions apply to different areas of research. For those interested in disease pathogenesis, Systems Biology may be defined as an information-rich discovery process that analyzes biological systems and their behavior as regulatory networks. Studying these networks in multiple relevant tissues and associating them to clinically relevant data can identify the complex biological patterns that underlie disease onset and progression. To restate this in terms of DKD research, Systems Biology uses a set of experimental tools and computational approaches that comprehensively identify networks of molecular changes that occur in patients with DKD compared to normal individuals and focuses when possible on changes in individual kidney cells or complexes of interrelated kidney cells. The analysis of these altered networks is used to identify associations among the many molecular changes that best predict and are likely to enhance disease progression or amelioration. Pragmatically, these studies are often restricted to one "scale" of molecular analysis (eg, transcriptomics focusing on gene expression phenotypes) although they may combine several scales, so called "multiscalar" or "multiomics" approaches (Fig 1), which often give the most powerful results. By their nature, systems biological studies are often hypothesis generating, not hypothesis testing. While not an issue for biomarker discovery, potential mechanisms of disease and therapeutic targets identified by systems biological approaches need to be validated by more conventional experimental methodologies. Examples of how systems biological approaches can be interwoven with more conventional experimental studies are noted in the following sections on DKD biomarkers and targets for therapy.

A number of systems biological reports on human DKD have been published over the past decade or more. Many of these report transcriptomic analyses examine the changes in gene expression in diabetic kidney tissues.

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A number of epigenomic, proteomic, and metabolomic analyses have also been reported. In the following sections, examples of each of these approaches will be described. This review is restricted to studies that have used human tissues and that demonstrate how systems biological approaches can move the field forward. It is not a systematic review. Moreover, we have not reviewed DKD genetic studies, as these have been extensively reviewed relatively and recently,[4] and no major advances have occurred in this area since that publication. The following examples have been chosen for illustrative purposes only, not because we believe they are definitive.

THE FUNDAMENTAL ROLE OF BIOINFORMATICS IN SYSTEMS BIOLOGICAL STUDIES

As noted previously, Systems Biology is inherently computational. The associations and connections in genome-wide data sets can only be made with computational tools, and the better and more sophisticated the computational tools, the better and more sophisticated the systems biological analysis. As will be noted in the following sections, many of the initial systems-wide studies utilized quite simple analytic tools and therefore provided little new insight into disease processes, though were important as proof-of-principle studies. Later studies have utilized a panoply of computational tools and have often generated specific tools to lead to the most meaningful observations and discoveries. We have emphasized these computational aspects in the following sections.

CLINICAL SUMMARY

- Systems biological approaches examine the network of all molecular changes that occur in a disease process instead of focusing on a single abnormal molecule (eg, gene or protein) or pathway.
- Diseases such as diabetic kidney disease (DKD) that involve multiple cell types in the kidney; result from multiple metabolic, inflammatory, and signaling changes; and evolve over years or decades are well suited for systems biological investigation.
- A major advantage of systems biological investigation is that it can start with investigation of DKD in humans and does not initially rely on models that generally fail to fully represent the human disease process.
- New biomarkers of progressive DKD and molecular targets for the treatment of DKD have been identified by systems biological approaches.

CLINICAL COHORTS AND BIOBANKING: THE CRITICAL UNDERPINNING FOR CLINICALLY PERTINENT DIABETIC KIDNEY DISEASE STUDIES

Because animal and cell culture models have only partly replicated the molecular phenotype found in humans with DKD and because diabetic animals do not develop progressive DKD, it has become imperative for meaningful DKD research to utilize human phenotypic data as a benchmark. This is especially true for systems biological studies which synthesize data from all molecular changes in the examined tissue or cell type and reflect species-specific pathways which, if taken from models, do not always overlap with those in humans.[5] Thus, a critical underpinning for systems biological studies of DKD has been the establishment and availability of long-standing cohorts of patients with CKDs including DKD. Especially important for this work has been the availability of carefully obtained and preserved kidney biopsy, blood and urine samples paired with detailed longitudinal clinical information acquired after kidney biopsies from participants in the cohorts. These have allowed identification of molecular pathways or molecules that best predict progression of DKD or are likely to be therapeutic targets without having to follow patients for years in the future. There now exist multiple CKD registries accompanied by large well-curated biobanks with multiple types of samples (including plasma, urine, and kidney tissue) that can be used for molecular analysis.

SYSTEMS BIOLOGICAL APPROACHES TO DEVELOP NEW DIABETIC KIDNEY DISEASE BIOMARKERS

Noninvasive molecular biomarkers with better sensitivity and specificity are urgently needed for the early diagnosis of DKD as well as identification of patients who are most at risk of disease progression. An ideal DKD biomarker would be easily detectable in body fluids such as plasma or urine, would represent diverse molecular mechanisms that underlie DKD pathogenesis, and would be kidney specific in order to eliminate effects of extrarenal sources. Most useful biomarkers are either proteins or metabolites, and unbiased proteomic and metabolomic approaches have helped discover a number of new potential biomarkers. Although none of these DKD biomarkers have been fully validated, several promising candidates have been discovered by these methods.

Transcriptomic Studies

A study from our group illustrated the feasibility of using transcriptomic data from human kidney biopsies to identify critical kidney-specific pathophysiologic molecules based on alterations in gene expression and then to test whether the protein products of these mRNAs could serve as urinary biomarkers to predict CKD progression.[6] Following this strategy, urinary epidermal growth factor (uEGF) was identified as an independent predictor of estimated glomerular filtration rate (eGFR) slope and of the composite CKD progression end point of ESRD or 40% reduction of baseline eGFR after adjusting for age, sex, baseline eGFR, and albumin-to-creatinine ratio. Moreover, uEGF added improved prognostic accuracy to eGFR and ACR in predicting progression in 3 separate CKD cohorts from different parts of the world. Although there was no specific DKD cohort included in this study, DKD patients were highly represented in one of the cohorts, and 135 CKD patients with diabetes, 70 of whom had biopsy-proven DKD contributed to the final results. A subgroup analysis of these diabetic patients also revealed a strong correlation between uEGF and eGFR or eGFR slope,[6] supporting the prognostic value of uEGF in