A Transcriptional Map of the Renal Tubule: Linking Structure to Function

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Differentiated organ function is critically dependent on the coordinated expression of a specific set of genes. Nephron segment–specific genes define the key functional characteristics of the kidney. Manual microdissection of the kidney into distinct tubular segments has provided unique insights into the function and failure of the kidney.1

The initial studies into the electrophysiology and biochemistry of microdissected tubular segments have subsequently been expanded using the tools of molecular biology to capture the molecular components defining the tubular microenvironments. Early studies focused on the gene expression of specific molecules of interest and were greatly facilitated by the development of ultrasensitive reverse transcription polymerase chain reaction.2 As transcriptional profiling techniques matured, more genome-wide approaches could be deployed on microdissected tissue. The landmark study from Chabardès-Garonne et al. used serial analysis of gene expression (SAGE) to define transcript maps from the main nephron subunits.3 Subsequent studies used predominantly Affymetrix-based hybridization platforms to generate expression profiling of glomeruli and tubular segments from animal models and human tissues in health and disease. These studies allowed transcripts to be catalogued to the main nephron units to be catalogued, but still had limits to the sensitivity and spectrum of transcripts detected. The introduction of next-generation sequencing technologies to transcriptional profiling of RNA (RNA-seq) now allows the transcriptome to be captured with unprecedented depth and sensitivity. In addition, RNA-seq can detect the complexity of alternative spliced transcripts in a sample. This is of particular relevance for tissue compartment-specific regulation, as the majority of multiexon genes in humans undergo alternative splicing to increase the functional diversity of protein species4 and are not comprehensively detected by most microarray platforms.5

In this issue of the Journal of the American Society of Nephrology, Mark Knepper and his team report the most


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comprehensive analysis of nephron segment–specific gene expression to date. Combining long-standing expertise in generating nephron segments of high purity with deep RNA-sequencing protocols allowed more than 8000 transcripts and their specific regulation in 14 structurally definable tubular subunits to be captured. The sequencing protocol allowed not only for the quantification of RNA abundance of known genes, but also for the description of nephron segment–specific alternative transcript expression.

HOW CAN THE NEPHRONRNASEQ DATA SET IMPACT OUR UNDERSTANDING OF RENAL PHYSIOLOGY?

So far we have defined tubular subunits by their structural and functional properties, many of which were discovered in isolated tubular segments after microdissection. However, functional characterization was targeted toward specific molecules of interest and constrained by limited accessibility to some tubular segments. The genome-wide RNA profiles reported by Lee et al. now allow cellular functions to be defined in a specific tubular segment based on the available transcripts to this segment. However, an inverse approach is also possible. Similarities among conventionally defined tubular segments can be established based on their transcriptome repertoires, establishing shared molecular machinery and relationships between tubular segments, which, up to now, have not been apparent. Transcript-based clustering of the 14 tubule segments studied defines six subgroups. Determining the transcripts responsible for the molecular subgroups can help to define the distinct functional properties of tubular compartments in an unbiased manner, stimulating further mechanistic studies. Examples provided by Lee et al. include the inference of specific metabolic capabilities available to distinct tubular segments based on the expressed transcripts. For example, the authors deduced, based on the expression pattern, that ATP production in the thin limbs is based on glucose metabolism, similar to more distal nephron segments, but quite different to the neighboring proximal tubules. The transcriptional data show a lack of key glycolytic enzymes in the proximal tubular compartment, consistent with prior knowledge.

The detection of tubular segment–specific transcription factors allows these factors to be associated with their potential target genes specifically expressed in those segments. These coregulation matrices of nephron segment–specific transcriptional functional elements can be of significant relevance both toward our understanding of developmental processes and the regulation of these elements in disease, as has been shown for glomerular data sets.

HOW CAN TUBULAR SEGMENT–SPECIFIC EXPRESSION DATA SETS IMPACT OUR UNDERSTANDING OF RENAL DISEASE?

Tissue compartment–specific transcriptional programs define the final stages in organ development and the mature function of all complex organisms. Alterations in the functions of genes with cell compartment–specific expression patterns are widely believed to be responsible for tissue-specific disease manifestations. Inherited diseases, in particular, are frequently caused by mutations in genes with tissue-restricted transcript patterns. Mutations in tissue-restricted transcripts often do not cause early embryonic lethality, rather the disease manifests at later developmental stages when the gene is expressed and its function becomes critical for a specific tissue. In the study of hereditary tubular diseases, next-generation exome sequencing technologies are identifying groups of putative causal genetic variants in very small pedigrees at an ever-increasing rate. However, as multiple candidate genes are often found, strategies to prioritize these genes for further analysis are critical. The NephronRNAseq data set provided by Lee et al. allows candidate genes to be efficiently mapped into defined renal tissue compartments. This facilitates the candidate gene prioritization process as the spatial and functional context of each candidate gene can be evaluated for a link to the observed clinical trait. The inverse approach might also be feasible, i.e., to test the tubule segment–specific genes for association with the disease in families where a hereditary disease phenotype can be assigned to a specific tubular tissue compartment.

For acquired kidney disease, the search is still ongoing to identify specific and robust biomarkers that are capable of capturing the loss of renal function. The disease markers currently used, GFR and proteinuria, are nonspecific and capture primarily hemodynamic and glomerular functions. Supplementation those parameters with markers reflecting specific tubular functions might allow detection of tubular dysfunction at an earlier time point. Proteins encoded by tubular segment–specific transcripts may be detected in plasma and/or urine; therefore providing noninvasive means thereby allows tubular compartment–specific organ function to be measured. In contrast to ubiquitously expressed molecules involved, for example, in fibrosis and inflammation, tubular-specific biomarkers are less likely to be confounded by extrarenal processes and should provide superior diagnostic specificity. Finally, tubular-specific molecules with a role in disease initiation or progression can be starting points for tubular-targeted therapeutic approaches with the potential for high tissue efficacy while reducing off-target effects.

To facilitate the broad applicability of the data set generated, the authors have not only deposited their data sets in gene expression omnibus, but also provide a searchable interface for broad use by the renal research community (https://helixweb.nih.gov/ESBL/Database/NephronRNAseq/index.html). The NephronRNAseq database will allow the integrating of the tubular segment–specific transcripts with further, complementary information sources. The NephronRNAseq data can, for example, be integrated with protein tissue localization provided by the Human Protein Atlas, which has established immune-histochemical staining patterns for more than 8000 proteins and provides high-resolution renal tissue stainings. The specificity of the staining patterns can now be cross-validated with the corresponding
transcriptional data from NephronRNAseq. Disease associated tubular-specific regulation can be determined by cross-referencing with expression data sets from renal biopsy tissue, using online data mining and analytical tools like nephromine.org. Lee et al. point specifically toward abundant and selective expression of defensin-ß1 in the collecting duct segments. Its expression might be considered as part of the antibacterial innate immune defense of the collecting duct, but interestingly we found defensin-ß1 downregulation in a subset of renal disease (diabetic nephropathy, immunoglobulin A nephropathy and lupus nephritis) in Nephromine, suggesting a regulation of this transcript in noninfectious renal diseases.

Steady-state mRNA levels are only one element defining the function of a tubular segment. Multiple additional layers of regulation are present and await analysis following the approach described in this issue of the journal for mRNA. A further frontier is the cellular heterogeneity present inside the tubular segment. This heterogeneity can be either addressed by in silico deconvolution\(^1\) or ex vivo by single-cell transcriptional analysis, a currently emerging technology applied successfully in other organ systems.\(^1\) The NephronRNAseq study presents a critical step forward toward a comprehensive understanding of the structural, functional, and molecular determinants of differentiated tubular function in health and disease.

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DISCLOSURES

None.

REFERENCES


A Friend in Need: Activated Protein C Stabilizes YB-1 during Renal Ischemia Reperfusion Injury

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AKI is associated with a rapid decline in renal function and high mortality rates. Furthermore patients that survive an acute illness have a high risk of developing ESRD from concomitant or aggravated CKD.\(^1\) Ischemia and reperfusion injury (IRI) is a leading cause of AKI. In the ischemic kidney decreased delivery of oxygen and nutrients results in tissue hypoxia and microvascular dysfunction while subsequent reperfusion amplifies inflammatory activation and cell death programs.\(^2\)

Current treatment of AKI is mainly supportive in nature as there is a lack of effective pharmacologic intervention. Despite

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