Mini-symposium

RNA-seq Profiling of Transcriptomes Along the Nephron

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The function of each renal tubule segment depends on the genes expressed therein. An unbiased, comprehensive profiling of steady-state mRNAs in tubular epithelia is an essential step toward a systems understanding of kidney functions in health and disease. High-throughput methods used for global profiling of gene expression in the nephron, such as microarrays and tag-based assays, have shown low sensitivity and high false positivity, thereby limiting the usefulness of these methods in transcriptomic research. Deep sequencing of RNAs (RNA-seq) achieves highly sensitive and quantitative transcriptomic profiling by sequencing complementary DNAs (cDNAs) in a massive, parallel manner. In this method, RNA species of interest are converted to adapter-ligated cDNA libraries for sequencing. The reported DNA sequences are mapped to the reference genome and the expression of an mRNA species is quantified as the number of sequences mapped to an annotated or de novo transcript.

Here, we used a modified RNA-seq technique coupled with classic renal tubule microdissection to comprehensively profile gene expression in each of 14 renal tubule segments from the proximal tubule through the inner medullary collecting duct of rat kidneys. Each renal tubule segment was collected from collagenase-digested rat kidneys by anatomical and histological criteria. After cell lysis, polyadenylated mRNAs were captured by oligo-dT primers and processed into adapter-ligated cDNA libraries that were sequenced using an Illumina platform. Transcriptomes were identified to a depth of ~8,000 genes in microdissected renal tubule samples (105 replicates in total) and glomeruli (5 replicates). Manual microdissection allowed a high degree of sample purity, which was evidenced by the observed distributions of well-established cell-specific markers. The main product of this work is an extensive database of gene expression along the nephron provided as a publicly accessible webpage (https://helixweb.nih.gov/ESBL/Database/NephronRNAseq/index.html). The data also provide genome-wide maps of alternative exon usage and polyadenylation sites in the kidney. We illustrate the use of the data by profiling transcription factor expression along the renal tubule and mapping metabolic pathways. This map of transcriptome along the nephron allows cellular functions to be defined and investigated in specific tubule segments and serves to generate testable ideas for hypothesis-driven studies. Future investigation into heterogeneity of gene expression within the same tubule segment at single-cell level will enhance our understanding of kidney functions.