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Urine Single-Cell RNA Sequencing in Focal Segmental Glomerulosclerosis—Hope for the Future

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Focal segmental glomerulosclerosis (FSGS) is a histologic pattern of injury rather than a disease. It is defined by the presence on kidney biopsy of sclerosis in parts of some glomeruli under light microscopic analysis. FSGS is mechanistically associated with injury to podocytes, terminally differentiated visceral epithelial cells with a very limited capacity to regenerate, that form the final barrier to urinary protein loss. Each human glomerulus is estimated to have 500-600 podocytes, and their loss by genetic, immunologic, metabolic, hemodynamic, and other insults leads to uncovered areas of glomerular basement membrane, ballooning of the capillary loop, synechia attachment to Bowman's capsule, and the development of FSGS. Seminal work by Wharram *et al.*[1] has shown that targeted podocyte depletion in a rodent model was sufficient to cause FSGS, where podocyte loss above a 40% threshold was associated with sustained high-level proteinuria and decreased kidney function. The traditional classification of FSGS was based on histologic variants—collapsing, tip, perihilar, cellular, and FSGS not otherwise specified. Unfortunately, the histologic variants cannot differentiate primary from secondary disease.[2] With advances in genomics and molecular biology bringing hope for much needed precision therapeutics, better ways to classify FSGS have been sought.

The most recently published 2021 Kidney Disease: Improving Global Outcomes guidelines highlight the heterogeneous nature and difficulty involved in fully accounting for the myriad underlying etiologies that give rise to these histologic findings.[3] The Work Group proposed classifying FSGS into 4 categories. Primary FSGS, which could be reasonably attributed to a circulating permeability factor, with a high risk of recurrence after transplantation, is characterized clinically by nephrotic syndrome and histologically by diffuse podocyte foot process effacement on electron microscopy. Genetic FSGS occurs where FSGS lesions develop in patients with disease-causing mutations in genes that encode podocyte or basement membrane proteins. Secondary FSGS is so designated when FSGS lesions are detected in the setting of known contributing etiologies such as viral infections (e.g., HIV, SARS-COV-2 with APOL1 high-risk genotypes), drugs (e.g., anabolic steroids, mTOR inhibitors), or adaptive changes associated with reduced (e.g., age-related FSGS, sickle cell disease, reflux nephropathy) or normal (e.g., obesity, other primary or systemic conditions) nephron number. FSGS of undetermined cause, is the proposed diagnosis of exclusion for clinical scenarios where genetic or identifiable secondary causes are absent and there is no diffuse foot process effacement on electron microscopy in a patient with FSGS lesions seen on light microscopy. Updated classification systems are certainly welcome as nephrology enters the precision medicine era. Evidence to date suggests that patients diagnosed with what is now referred to as primary FSGS should be treated with available immunosuppressive agents while scientific efforts to characterize circulating permeability factors and develop targeted interventions are prioritized. Noninvasive, scalable approaches to diagnose and stratify FSGS also represent an important unmet need.

RNA sequencing (RNA-seq) is a genomic application for the detection and quantitative analysis of mRNA. Classically, these transcriptomic studies provide valuable insights into cell and tissue function where levels of gene expression are used as a proxy and useful correlate for encoded protein and cellular traits. An important limitation, however, is that

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traditional bulk RNA-seq experiments are performed on whole organs or tissues, thereby failing to capture cell to cell variations in gene expression and regulatory changes. Single-cell RNA-seq (scRNA-seq) facilitates unbiased genome-wide transcriptomic profiling of individual cells that are first isolated for individual RNA capture. Single-cell analysis has been successfully used to generate a map of gene expression in most kidney cells, facilitating a transition from defining cellular properties by morphologic characteristics to one based more objectively on gene expression.[4] Single nuclear RNA-seq, where nuclei are isolated from cells, is an alternative to scRNA-seq and is attractive because good quality nuclei can be isolated from snap-frozen samples and can often be captured more completely. Early human diabetic nephropathy gene expression changes have been defined by single nuclear RNA-seq.[5]

There have been some recent attempts to apply single-cell technology to urine samples, including a recent study in patients with biopsy-proven diabetic kidney disease and pooled controls that confirmed that most kidney cells can be identified in the urine.[6] scRNA-seq has also been used to characterize the urinary sediment of patients hospitalized with COVID-19[7] and to define the cellular profile of healthy urine.[8] In this issue of *KI Reports*, Latt *et al*.[9] used a pilot approach to test the utility scRNA-seq in characterizing the urinary transcriptomic profile of 12 subjects with FSGS. The findings reveal the presence of immune cells, podocytes, myofibroblasts, and tubular cells with distinct expression profiles. They identified a podocyte cell cluster expressing previously reported WT1, PLA2R1, SYNPO, and IGFBP7 markers. Interestingly, urinary podocytes showed loss of canonical NPHS1, NPHS2, and PODXL markers and high expression of epithelial-to-mesenchymal and parietal epithelial cell markers. These findings support the theory of podocyte dedifferentiation associated with loss into the urinary space.

The authors identified 2 urinary monocyte subtypes: M1 (TIMP1 and IL1B expression) and M2 (APOE and APOC1 expression) that are also highly expressed in myeloid subpopulations of kidney immune cells in lupus nephritis. Relevant to ongoing FSGS research, inflammatory monocytes were found to express high levels of PLAUR, the gene encoding suPAR, a candidate circulating permeability factor. They found that APOE was the most significantly up-regulated gene in FSGS monocytes, with the expression of APOC1, SPP1 (encoding the immune modulator osteopontin), and several metallothionein genes also increased. Beyond the identification of epithelial-to-mesenchymal and immune gene expression signatures, the study also used analysis of ligand-receptor interactions to reveal that tumor necrosis factor signaling is involved in crosstalk between immune and renal epithelial cells. Among these, TWEAK/Fn14 and TRAIL/DR5, which already been reported to induce apoptosis, chronic inflammation, and fibrosis, are potentially the most prominent interaction.

The study has several limitations, including the acknowledged small patient sample size and absence of healthy subjects or subjects with minimal change disease. Given the aforementioned heterogeneity in FSGS, it is important to recognize, therefore, that the analysis performed was on patients with essentially different underlying diseases. Correlation with biopsy findings or defined clinical features with respect to kidney function and level of albuminuria was therefore not possible. From a technical perspective, cells with <100 genes were excluded from the analysis. This is a lower threshold than the standard 200 to 400 genes per cell but was presumably done to overcome the smaller number of cells in the urine of patients with FSGS compared with other kidney diseases.

Nonetheless, this study has notable clinical and scientific implications. From a mechanistic perspective, the findings suggest that monocytes could be a source for circulating permeability factor(s) that can be detected in the plasma and urine. In addition, the findings are consistent with another scRNA-seq study in lupus nephritis that reported that gene expression of immune cells in urine were highly correlated with their kidney tissue expression, suggesting that urine could potentially serve as a surrogate for kidney biopsies.[S1] Given the obvious noninvasive nature of urine collection, this scRNA-Seq approach in larger studies in well characterized patients and appropriate controls could yield important diagnostic and prognostic signatures, particularly for primary FSGS.

DISCLOSURE

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SUPPLEMENTARY MATERIAL

Supplementary File (Word)

Supplementary Reference.

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