selection of the appropriate animal model significantly influencing overall success. A better understanding of similarities and differences of cellular crosstalk between kidney cells in animal models therefore is also crucial for selecting the right model system to study effects of compounds under development on dysregulated molecular processes. Raghubar *et al.*[8] used spatial transcriptomics to compare receptor ligand interactions between human and mouse kidney tissues. They highlight significant changes in expression of genes being linked to energy production and metabolic processes when comparing data across the 2 species.

Results from scRNA-seq studies in addition also hold the potential to provide input for organ-on-a-chip experiments that still pose an efficient way to investigate novel compounds regarding toxicity and impact on kidney cell types *ex vivo*.[9] In light of all these new findings from the data-driven studies in animal models as well as in human samples, however, optimal setup and fine tuning of kidney-on-a-chip experiments might be even harder than initially anticipated.

Overall, these are exciting times for the field of nephrology with the new technologies opening doors to get a better understanding of disease pathophysiology and structure-function relationships. Because of the number of highly specialized cell types within the kidney, nephrology may be one of the medical fields profiting the most. We can at this point only speculate whether this is causally linked to the overproportional rise in impact factors of major nephrological journals as compared with other areas of medicine. Nephrological researchers in any case nowadays have a number of tools at hand and data available to derive hypotheses on new biomarkers, affected molecular pathways, or novel treatment options. Pathologists in addition are expected to benefit from these new technologies and will be supported in the assessment of a kidney biopsy specimen.

The study by Stefansson *et al.* on the association of kidney cell–cell crosstalk with hyperfiltration along with the other discussed studies represents the beginning of a new era in understanding the impact of cell–cell interactions on disease development and progression on a systematic level in nephrology.

DISCLOSURE

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Urinary single-cell transcriptomics: a promising noninvasive method for assessing acute kidney injury

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Noninvasive methods for obtaining intrarenal information are required to understand the mechanism of acute kidney injury (AKI). Klocke *et al.* explored the feasibility of using urinary single-cell RNA sequencing in assessing human AKI. Urine samples from patients with AKI included tubular epithelial cells with injury-related dedifferentiation and adaptive phenotypes, which could reflect kidney tissue damage. Thus, urinary single-cell RNA sequencing would provide new insights into human AKI, leading to the identification of novel biomarkers and therapeutic targets.

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Acute kidney injury (AKI) is a major health issue with no effective targeted therapies. AKI survivors are prone to chronic kidney disease (CKD), increasing the risk for renal replacement therapy, cardiovascular events, and high mortality.[1] The mechanism of the AKI-to-CKD transition has not been fully elucidated partly

because it is difficult to obtain the intrarenal information during AKI. Although kidney biopsy is critical for the precise diagnosis of kidney disease, it is not feasible for patients with AKI because major bleeding complication risk is extremely high in patients with AKI compared with patients with normal kidney functions.[2] Therefore, it has been attempted to use noninvasive urinary information in the diagnosis of AKI. Urinary biomarkers such as liver-type fatty acid binding protein, neutrophil gelatinase–associated lipocalin, and kidney injury molecule 1 are known to reflect AKI prognosis to some extent.[3] However, these urinary biomarkers cannot explicitly reflect what is occurring in the kidney tissue of patients with AKI.

In recent studies using mouse AKI models, many important findings were obtained through single-cell transcriptomics of kidney tissues. Single-cell RNA sequencing (scRNA-seq) of mouse AKI tissues demonstrated that repairing processes of injured proximal tubular cells are heterogeneous. Although most tubular cells are fully regenerated after injury, some injured cells fall into failed-repair states.[4] These failed-repair tubular cells are considered to be involved in inflammation and fibrosis in kidney tissues, leading to CKD progression.[5] Thus, the assessment of heterogeneous tubular cell states is critical for understanding the mechanism of the AKI-to-CKD transition.

The examination of urine sediments is a well-established practice in daily clinical settings. Although most cells in the urine are shed from urinary tracts, kidney parenchymal cells also exist in the urine sediments especially from damaged kidneys.[6] However, the assessment of urine sediments is currently based on morphologic observations, which cannot analyze the heterogeneity of tubular epithelial cell states.

To address this issue, Klocke *et al.*[7] explored the feasibility of using urinary scRNA-seq in assessing human AKI. They analyzed 42,608 single-cell transcriptomes of 40 urine samples from 32 patients with AKI. Although healthy control urinary cells in the previous study[6] consisted almost entirely of urogenital tract cells, urine samples from patients with AKI included kidney parenchymal cells and immune cells as well as urogenital tract cells, enabling the analysis of damaged tubular epithelial cells. In the focused analysis of kidney parenchymal cells, most tubular epithelial cells from urine samples of patients with AKI did not express characteristic segment markers, and instead showed injury-related dedifferentiation and adaptive phenotypes. To what extent these urinary cells reflect kidney pathophysiology was investigated by mapping the urinary single-cell data onto the reference atlas constructed from single-cell data of human AKI postmortem biopsy samples.[8] The analysis showed that urinary tubular cells with injury-related transcriptomes share similar characteristics with injury-related tubular cells from biopsy samples. However, the urinary tubular cells were biased toward medullary and distal nephron segments, suggesting that cells located in the distal part are more prone to final urinary excretion compared with proximal tubular cells. Although the urinary adaptive cell states resemble the repairing process of proximal tubular cells in mouse AKI models, these cells were not derived from proximal tubules but from the thick ascending limb according to their time course analysis (Figure 1). This result might be due to the comparatively low abundance of proximal tubular cells in the urinary samples. Finally, the alteration of urinary tubular epithelial cell abundance and cell-type proportions was analyzed over time after AKI. The urinary cell abundance was the highest during days 6–10 after AKI. Also, tubular cells with injury, inflammation and tissue rearrangement markers and with oxidative stress markers were especially increased during days 6–10. These results demonstrate the temporal shifts in the urinary cell signature after AKI. Thus, the authors concluded that urinary scRNA-seq provides noninvasive, unprecedented insights into cellular processes underlying AKI.

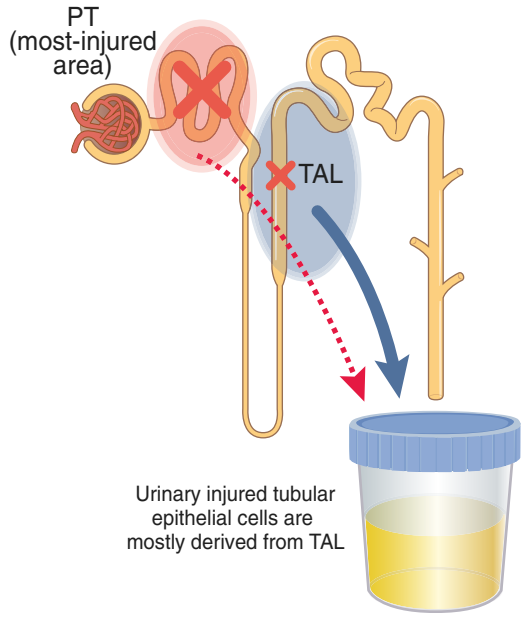

Figure 1 | The origin of tubular epithelial cells in the urine of patients with acute kidney injury. Urine samples from patients with acute kidney injury included tubular epithelial cells with injury-related dedifferentiation and adaptive phenotypes. However, these cells were mostly derived from the thick ascending limb (TAL), not from proximal tubules (PT). Future studies are needed to confirm whether urinary injured cell states originated from distal segments really reflect intrarenal information including damaged PT.

The most important finding of this study is that urine samples from patients with AKI include tubular epithelial cells with injury-related transcriptomes. Analysis of time-dependent changes in the urinary adaptive cell states would provide a better understanding of the human AKI-to-CKD transition, leading to the identification of critical biomarkers or therapeutic targets. Especially, future clinical investigations may align longitudinal urinary scRNA-seq with clinical situations in which AKI is expected to occur, such as cardiac surgery, anticancer drug therapy, and kidney transplantation. Correlation analysis of single-cell transcriptomes with patient outcome may identify novel cell types/states or cell type–specific markers that could facilitate prognosis and guide disease management. However, some issues remain to be addressed before urinary scRNA-seq can be widely used in clinical settings.

First, the tubular epithelial cells in urine samples were biased toward medullary and distal nephron segments. Many preclinical studies showed that damage to proximal tubular cells plays a crucial role in the AKI-to-CKD transition.[4] Proximal tubular cells are prone to acute environmental change such as ischemia and toxic substances because they are highly energy-demanding due to the need for reabsorption of glucose and sodium to maintain homeostasis of body fluids.[9] However, in the present study, proximal tubular cells were rarely detected in urine samples probably due to the anatomical location. The pseudotime trajectory analysis showed that urinary injured cell states were mostly derived from the thick ascending limb (Figure 1). Thus, it is important to confirm whether urinary injured cell states originated from distal segments really reflect the intrarenal information including damaged proximal tubular cells. Ideally, urinary scRNA-seq data should be compared with kidney tissue data from the same patients. However, as it is difficult in a clinical setting, more urine samples from patients with AKI should be collected and cautiously compared with clinical manifestation in future studies, which would unravel the clinical significance of urinary injured cell states.

Second, urine sample preparation methods should be improved to perform large-scale studies. Fresh urine samples are immediately used in the current study because cell sorting by the flow cytometric approach is needed to obtain viable cells. Because of this technical limitation, the authors could not collect urine samples at desired time points to perform the detailed analysis of time-dependent urinary changes. Therefore, establishing the standardized method for sample preservation is needed to make the best use of urinary scRNA-seq in assessing human AKI.

Finally, it should be investigated whether urine concentrations would affect cell state and number detected in the urine, independent of kidney tissue damage. In the current study, oligo-to-anuric AKI cases were excluded from analysis, representing a major limitation when using urine samples as a diagnostic tool. Even if diuresis is preserved, urine volume and concentration keep on changing during AKI. In addition, some patients with AKI would be given diuretics, which might independently affect urinary cell state and number. If urine samples contain very few cells, rare cell types or states might be undetected, biasing the analysis. Thus, it is important to know the effect of urine concentrations and use of diuretics on urinary cell signature of patients with AKI, considering the clinical application in the future.

In conclusion, this landmark study showed the feasibility of using urinary scRNA-seq in assessing human AKI. As tubular cells with injury-related transcriptomes are included in urine samples of patients with AKI, intrarenal information could be noninvasively obtained by analyzing these cells. Although improvement of the method for urine preservation is required for performing large-scale studies, urinary scRNA-seq would provide new insights into human AKI, leading to the identification of new biomarkers and therapeutic targets in the future.

DISCLOSURE

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