Advances in Renal Cell Imaging

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Summary: A great variety of cell imaging technologies are used routinely every day for the investigation of kidney cell types in applications ranging from basic science research to drug development and pharmacology, clinical nephrology, and pathology. Quantitative visualization of the identity, density, and fate of both resident and nonresident cells in the kidney, and imaging-based analysis of their altered function, (patho)biology, metabolism, and signaling in disease conditions, can help to better define pathomechanism-based disease subgroups, identify critical cells and structures that play a role in the pathogenesis, critically needed biomarkers of disease progression, and cell and molecular pathways as targets for novel therapies. Overall, renal cell imaging has great potential for improving the precision of diagnostic and treatment paradigms for individual acute kidney injury or chronic kidney disease patients or patient populations. This review highlights and provides examples for some of the recently developed renal cell optical imaging approaches, mainly intravital multiphoton fluorescence microscopy, and the new knowledge they provide for our better understanding of renal pathologies.

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The currently used applications and further development of highly innovative future next-generation cell and tissue imaging technologies using novel multiplexed fluorescence as well as label-free nondestructive microscopy techniques are essential for the highly precise quantitative and automated (unbiased) analysis of kidney structure and function. These include both research and clinical uses of the living or fixed kidney tissue, and are especially relevant and important for the single-cell–level investigation of human renal nephrectomy and biopsy specimen. The need for high-precision cellular imaging as a diagnostic tool, along with the use of molecular-level omics approaches, is well justified by the recognition of individual variability in the cellular and molecular pathomechanism of human diseases including acute kidney injury (AKI) and chronic kidney disease (CKD).[1,2] In fact, the National Institutes of Health recently launched its precision medicine initiative,[3] which focuses on accelerating the development of individualized cell and molecular mechanism–based targeted treatments not only for cancer, but other diseases as well. The translation of this initiative, and the already ongoing efforts in the renal field, are well illustrated by the most recent birth of the Kidney Precision Medicine Project (KPMP), funded and overseen by the National Institutes of Health/*National Institute of Diabetes and Digestive and Kidney* Diseases.[4] Advanced renal cell imaging will be a key part of the KPMP, which aims to create a kidney tissue atlas, define disease subgroups, and identify critical cells, pathways, and targets for novel therapies.[4] For example, detailed analysis of the spatial distribution of single-cell states by highly multiplexed single-cell and molecular imaging with subcellular and three-dimensional resolution will be essential for building a kidney tissue atlas for future data mining and new therapeutic and biomarker development.

Next-generation imaging techniques set their foundation on current renal cell imaging approaches. Optical imaging techniques, including multiphoton microscopy (MPM), have been used and perfected throughout decades for the detailed and quantitative imaging of kidney structure and function.[5–9] Because of its submicron resolution and harmless, deep-tissue imaging capability,[10–12] MPM ideally is suited to

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perform renal cell imaging in the intact living kidney in vivo, or large volumes of fixed kidney tissue. Applications of MPM combined with tissue-clearing techniques of the human kidney showed diagnostic-level image quality that can be maintained through 1 mm of tissue, and therefore have great potential for increasing the yield of histologic evaluation of biopsy specimens.[13] Similar approaches are expected to be instrumental also for the efforts of the KPMP to build a kidney tissue atlas.[4] For intravital applications, MPM has solved a long-standing critical technical barrier in renal research to study several complex and inaccessible cell types (eg, the podocyte) and anatomic structures (such as the glomerular filtration barrier) in vivo in their native environment.[14–17] Various modalities for the quantitative MPM imaging of the basic parameters of kidney function (eg, single-nephron glomerular filtration rate [GFR], blood flow, tubular fluid flow, glomerular albumin leakage and tubular uptake, the intrarenal renin-angiotensin system, and so forth) have been reported and reviewed elsewhere.[11,18–24] Refinements of these techniques including successful fiberoptic delivery of photons and minimally invasive intravital endomicroscopy of the kidney may one day lead to human clinical diagnostic applications.[25]

This article focuses on renal cell imaging, highlighting recent advances in the quantitative visualization of the identity, density, and fate of both resident and nonresident cells in the kidney, and imaging-based analysis of their altered function, (patho)biology, metabolism, signaling in the rodent (research applications), as well as the human kidney (clinical diagnostic and translational applications). We review some of the recently developed renal cell optical imaging approaches and also provide practical examples with supporting new preliminary data for their potential applications, advantages, feasibility, and promise in further improving our understanding of kidney disease mechanisms and developing new therapeutic and diagnostic applications with the principle of kidney precision medicine.

SINGLE-CELL AND CELL LINEAGE IDENTIFICATION AND FATE TRACKING

The precise identification and analysis of resident and nonresident renal cell types within kidney tissue specimens are essential for studying disease pathobiology, histology, cell-to-cell interactions, therapeutic effects of drugs, clinical differential diagnostics, and so forth. Genetic labeling of renal cell types using fluorescent lineage tags and genetic cell fate tracking in animal models provided highly specific, positive identification of several renal cell types and their fate changes. For example, cell-specific, promoter-driven expression of monochromatic (eg, the green fluorescent protein [GFP]) or multicolor fluorescent tags (eg, the two-color, membrane-targeted tandem dimer Tomato or GFP[26] and the four-color Confetti construct of cyan, green, yellow, and red fluorescent proteins)[27] allowed the precise identification of glomerular cells including podocytes and parietal epithelial cells (PECs) in both mouse[14,16,28–31] and zebrafish models.[15,32] The use of these and similar cell identification research tools for genetic cell fate tracking purposes provided evidence for the presence of both motile and stationary podocytes and PECs,[14–16,31–33] podocyte clustering and migration to the parietal Bowman's capsule,[16,34,35] and the recruitment of podocytes from PECs.[28,33,36] In general, these studies captured the highly dynamic nature of cellular remodeling of the glomerular environment, especially under glomerular injury conditions including atubular glomeruli, unilateral ureteral obstruction, and the adriamycin nephropathy and cytotoxic IgG-induced podocyte injury models of focal segmental glomerulosclerosis.[16,17,28,33–36] In addition, the combination of multicolor genetic cell identification and fate tracking with serial MPM imaging of the same glomeruli in the same kidney and animal over several days is the only currently available technique that allows the investigation of the migration pattern and dynamics of the same single renal cells in the intact living kidney.[11,16,21,34,37,38] This approach has great potential for the unbiased study of the cellular and molecular mechanisms of endogenous tissue remodeling and repair after injury. Genetic cell fate tracking tools and approaches have been described for several developmental or injury-related renal progenitor cell populations and their lineages to study kidney regeneration, including glomerular epithelial cells (eg, Pax2 lineage),[28] proximal tubule cells (eg, Six2 and Sox9),[39–41] the distal nephron (eg, Lgr5),[42] and collecting ducts (eg, Aqp2, Slc12a3, and p63).[43,44] In addition, many different cell compartments of the renal interstitium and vasculature have been studied using similar tools including pericytes (eg, Foxd1, Coll1a1, and Gli1 lineage),[45–48] vascular endothelial cells (eg, Tie2),[21,49] and cells of the renin lineage (eg, Ren1d and Ren1c).[38,50]

In Figure 1, we show examples of intravital MPM imaging of less commonly studied renal cell types and genetic models. Multicolor labeling of cells of the renin lineage can identify individual cells of the classic vascular site (juxtaglomerular apparatus) as well as the novel tubular site of renin production in the connecting tubule-collecting duct system (Fig. 1A-C), consistent with earlier descriptions of renin expression and renin cell distribution.[51,52] The presence of cell labeling in the Bowman's capsule and glomerular mesangium (Fig. 1A and B) is in agreement with the findings that cells of the renin lineage are adult pluripotent