Perspective

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Imaging and spatial omics of kidney injury: significance, challenges, advances and perspectives

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Abstract: The kidneys are susceptible to a range of insults that can cause damage to them. Early diagnosis, timely prevention, and proper treatment are crucial for improving the outcome of kidney injury. However, the complexity of renal structure and function makes it difficult to reach the demand of early detection and comprehensive evaluation of kidney injury. No successful drug therapy caused by the elaborate pathogenesis mechanism network of kidney injury calls for a systematical interpretation in mechanism researches. Recent advances in renal imaging and omics studies have provided novel views and deeper insights into kidney injury, but also raise challenges in reaching a comprehensive cellular and molecular atlas of kidney injury. Progresses in imaging and omics of kidney injury are being made in various directions, with the initiative of construction a high-resolution structural atlas of kidney, dynamic and non-invasive evaluation of renal function, and systematic establishment of spatially resolved molecular atlas by transcriptomics and metabolomics. With the limitations of a single modality, novel multimodal integration technologies of imaging and omics are being attempted to achieve a systematic description of nephropathy mechanisms. Further extensive efforts in renal multimodal imaging and omics studies are extremely required to deepen our understanding on kidney injury in the context of diagnostic, mechanistic and therapeutic perspectives.

Keywords: kidney injury; imaging; omics; multi-modalities

Introduction

Kidney is a vital metabolic and excretory organ in the human body. Its high blood flow and vigorous excretion function make it vulnerable to various insult factors such as immunity, poison, ischemia, infection, and metabolic disorders, resulting in acute kidney injury (AKI) [1]. Previous research of our group demonstrated that the incidence of AKI caused by various reasons was about 20 %–50 % among high-risk inpatients [2]. Due to the low early diagnosis rate and the lack of effective therapeutic medications, the clinical management of AKI is highly challenging, with the mortality rate of hospitalized AKI patients kept high at about 25 %, and 50 % of the AKI patients did not fully recover their renal function and eventually transitioned to chronic kidney disease (CKD) [2].

Early diagnosis, timely prevention, and proper treatment of the pathogenic insults are crucial to improving the outcome of AKI. However, the complexity of tissue structure and physiological functions and the variety of pathogenesis on the large scale of acute kidney injuries make it extremely difficult to reach an early detection and a comprehensive evaluation of kidney injury, which greatly impedes the development of precision medicine for AKI. Therefore, constructing a systemic and panoramic evaluation system of kidneys is crucial for the improvement of clinical diagnosis and the development of new drugs [3]. Recent advances in renal imaging and omics studies with various scales or modalities have provided novel views and deeper insights into kidney injury. These bring us precious opportunities but also raise rigorous challenges in reaching the landmark

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of a comprehensive cellular and molecular atlas of kidney injury. Here in this article, we present our views on this booming and brand-new area in kidney injury studies.

Significance

Nowadays, the improvement of the capacity of clinical diagnosis and treatment is becoming a major clinical need.

Diagnosis

In the clinical field, four major demands for full achievement of early diagnosis, etiological diagnosis, prognosis evaluation, and the right timing of treatment decisions are increasing. However, the existing methods and strategies for AKI diagnosis, such as clinical imaging and biomarkers, are far from developed. Renal pathology is still the leading strategy and gold standard of diagnosis. However, clinical kidney biopsy is highly traumatic and limited in sampling. Excepting minor complications of transient bleeding, fever, hematuria, proteinuria and transient hypertension, major complications such as puncture of nearby organs or structures, infection near the biopsy site, development of arteriovenous fistula or pseudoaneurysm happen occasionally. Cases of nephrectomy or even death after renal needle biopsy are also factors that cannot be ignored in some extreme cases. As a result, it is not feasible for dynamic assessment of kidney injury or being performed in patients with severe conditions or with contraindications. Even after a successful and safe renal puncture, the low depth and small volume of biopsied samples disabled the pathologists from having a global view of the kidney and therefore may miss important disease information. These gave rise to a relatively low diagnosis rate and a limited precision of etiology identification [4]. In this case, it is in urgent medical need and of great clinical significance to develop a novel clinically practicable diagnosis strategy by imaging with high standard of non-invasion, dynamics and continuity.

Treatment

In the aspect of treatment, another sacred realm of kidney injury research is to interpret the elaborate mechanisms underlying the development and progression of long-lasting injury, since no successful drug therapy exists for AKI or reversing kidney fibrosis after so many years of mechanism study [1]. Besides the novel molecular targets are required to be identified urgently, their function in the time-series from the beginning to the end should also be elucidated clearly for a proper decision of treatment window. It is most meaningful to depict the mechanisms of kidney injury systematically, panoramically and comprehensively.

Opportunities

The rapid development of advanced technologies greatly facilitates cross cooperation and brings out many opportunities and novel insights into kidney diseases. Revolutionary technologies in kidney imaging and omics have made it possible to construct three-dimensional tissue maps with unprecedented spatial and molecular resolution. Medical cross-studies organized with these technologies are potential and promising approaches to removing obstacles to the full achievement of precision medicine for kidney diseases. This field is demonstrating powerful capabilities in meeting the growing diagnosis demands and solving complicated mechanism issues of renal diseases inch by inch.

Challenges

The number of renal studies in imaging and spatial omics is still largely limited due to several obstacles. At present, the main challenges of renal disease research to overcome are addressed in several aspects. (1) Complexity of renal tissue structure; (2) diversity of renal function; (3) complexity of pathogenesis of kidney diseases. The abundant cell types before and after injury are highly heterogeneous, and the spatial structure as well as segment variation composed by these cells is extremely complex. The pathological changes in kidney induced by various types of insults are more complex and exert differential effects on each cell type and segment resident in distinct spatial location, thus, giving rise to more complicated renal microenvironments. After injury occurred, renal tubular epithelial cells and vascular endothelial cells showed distinct degrees of injury, different subcellular structures alteration induced by heterogenous molecular mechanisms over time and space. Pathological changes such as infiltration of different subtypes of inflammatory cells in the mesenchyme and abnormal activation of different states of fibroblasts make the pathogenesis network more complex. In the chronic phase of renal injury, focal lesions and fibrosis sites arise, suggesting heterogeneous microenvironments composed of similar cell types. The same molecule or biological process might even play different protective or noxious roles in different stages of kidney injury [5].

Thus, these difficulties present great challenges and specific considerations related to renal imaging and spatial omics. (1) At the forefront are the challenges in restoring dynamic and continuous sample information with high quality. A miniaturized portable device must sacrifice temporal resolution and output modalities. (2) Limitations from a single modality and development of novel multi-modal integration technologies and algorithms. Integration of tissue structure, renal function and molecular information are of great importance to deal with the multifaceted microenvironments that give rise to the focal lesions in the end. (3) Difficulties in implementation of cellular resolution, field of whole kidney and high-throughput at the same time. A systematic profiling of whole kidney in detail to resolve heterogeneity and complexes of cellular and molecular spatiotemporal networks by imaging and omics depends upon the balance and development of large field, high resolution, depth and throughput. So far, imaging techniques commonly used in clinical practice, such as B-ultrasound, X-ray, computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography-computed tomography (PET-CT) are limited in studying kidney injury due to their low resolution. Imaging methods commonly used in pathology, such as optical microscopes, fluorescence microscopes and electron microscopes, could only be used for local imaging but are not suitable for cross-scale physiological and pathophysiological studies of the whole intact kidney [6]. At the same time, spatial omics based on sequencing retained depth and throughput superiority at the cost of losing field and resolution, while spatial omics based on imaging are advanced in field and resolution by losing the advantages of depth and scale. Even resorting to registration and 3D reconstruction algorithms, it is still not viable to reduce and resolve complex information of an intact mouse kidney. Therefore, for the time being, commonly used imaging and omics methods are not feasible to accurately capture lesions, unable to achieve cross-scale kidney 3D panoramic imaging, and therefore difficult to provide panoramic structure, function and molecular mechanism information of kidney injury.

Advances

Progress is being made independently in various directions in imaging and omics of kidney injury. They attempted to achieve the above goals from different single aspect, such as construction of a high-resolution structural atlas of kidney, dynamical assessment of vascular or metabolic function alteration in kidney injury, non-invasive evaluation of renal function of AKI patients and systematic establishment of spatially resolved molecular atlas by transcriptomics and metabolomics.

X-ray imaging

X-rays are less commonly used in kidney disease due to low soft tissue contrast and radiation concerns for CT, yet their non-invasive and dynamic advantages make them a potentially strong competitor. The application of micro-CT in kidney imaging still relies on the various contrast agents for better soft tissue contrast now. With the help of blood vessel contrast Microfil, it is possible to reconstruct vascular tree structure with 3 μm resolution and reveal the reduction of vascular density *in vitro* following injury. Besides contrast agents, another way of enhancing contrast is to use synchrotron radiation X-ray (SRX). With characteristics of strong penetration, high sensitivity, multi-scale resolution and low radiation dose, it is widely used in multi-scale, high-sensitivity, low-dose and non-destructive imaging of complete organs of humans and model animals. The 3rd generation of SRX by European Synchrotron Radiation Facility (ESRF) enabled hierarchical phase-contrast tomography (HiP-CT), which further gave rise to the first cross-scale scan of an intact human kidney [7]. Through combining the breakthrough of virtual histology (VH) image digital conversion technology, it allowed construction a panoramic nondestructive 3D pathology map of kidney. The utility of SRX-based HiP-CT may play a vital role in revealing the physiological microstructure, as well as its pathophysiological alteration in time series of kidney injury. Thus, X-ray imaging has the potential of construction a brand-new and revolutionary 3D pathology diagnosis system for kidney diseases, as well as a microstructure atlas for mechanism research.

Molecular imaging

Real-time and non-invasive *in vivo* monitoring of the molecular-level pathophysiological status within renal tissues is crucial for understanding diseases and developing therapeutic targets. Currently, commonly used clinical methods, such as non-invasive measurements of glomerular filtration rate (GFR), urine sediment analysis as well as invasive renal biopsy fail to achieve the goal. Molecular imaging techniques offer the potential to observe molecular processes and blood supply inside the kidney by injecting specific radioactive substances,

contrast agents, specific molecular probes or material tracers which are expected to provide more accurate and personalized functional information for the diagnosis and treatment of kidney diseases. Molecular imaging can utilize various imaging modalities such as MRI, PET-CT, single-photon emission computed tomography (SPECT) and fluorescence molecular tomography (FMT). Molecular renal probes (MRPs) are used for labeling early biomarkers of AKI, which enable long-term real-time imaging of multiple molecular events in the kidneys of live mice and further monitor the onset, progress and outcome of kidney injury [8]. Detailed molecular imaging probes in previous studies have been addressed elsewhere [9]. Besides molecular imaging, imaging based on advanced material is also rising and playing more roles in AKI diagnosis. Novel microbubbles tracked by super-resolution ultrasound (SRUS) imaging as they flow with the blood, also allowed for non-invasive *in vivo* evaluation of vascular function and structure following ischemia-induced AKI or various types of glomerular injury [10]. Focusing on spatial heterogeneity, cationized ferritin-enhanced magnetic resonance imaging (CFE-MRI) was used to examined the three-dimensional spatial distribution of nephron density and calculation of single nephron glomerular filtration rate (snGFR) [11]. Thus, the advanced molecular imaging facilitated the detection of focal lesions that are distributed irregularly, and make possible of the mechanism research behind. The booming of probe and material development have made molecular imaging expandable and more powerful in monitoring kidney injury precisely.

Spatial imaging omics

Single-cell omics such as RNA sequencing have tremendously accelerated the mechanism studies of kidney injury in the last decade, yet the failure of retaining spatial information of cells impeded the precise interpretation of microenvironment, spatial neighborhood and niche networks of kidney injury. The first spatial omics atlas of kidney injury was generated by the transcriptomics solution from the 10× Genomics Visium Spatial Gene Expression. Spatial molecular expression patterns of repair and injury response to ischemic insult could easily be defined and uncovered by spatial clustering analysis and identification of spatially variable features. Deconvolution of spatial atlas also provided a preliminary solution to detection of key cell-cell interaction pattern in the elaborate injured microenvironment [12]. Another spatial atlas by matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) gave the first metabolomics landscape of AKI. Combined with multiplexed immunofluorescence staining to label specific tubular segment or state, metabolic signature of region-specific cell types related to repair and maladaptive repair could be identified, and the trajectories of metabolic pathway alteration along the kidney injury progression were able to be determined [13]. However, in exchange, implementation of the large field of Visium and MALDI-MSI sacrifices the resolution of single cell, which is most precious for precise depiction of molecular signature, fate trajectories and interaction patterns between spatially variable cell types or even subgroups. At present, advances in spatial technologies are breaking the borderline between imaging and sequencing-based omics, which truly catalyze the birth of spatially single-cell resolved detection. Technologies of novel single-cell resolved spatial imaging and omics that are promising in kidney injury research were summarized (Table 1). As was displayed, without losing spatial resolution or large field of view (FOV), these technologies are gradually developed to have higher throughput and better compatibility with proteomics or metabolomics, no matter within the same slice or by sequential slices. Better compatibility with formalin fixation and paraffin embedding (FFPE) slices also makes possible of establishment of kidney injury cohort. This will greatly fill the huge gap of prognostic research in the field. Outcome-related cell populations, cell-cell interaction patterns and microenvironment signature will be uncovered to provide a clearer direction for mechanism research. In conclusion, with a better balance of depth, scale, throughput, field and resolution, application of spatial imaging omics in kidney injury will overcome the challenge of heterogeneity and complexity with less hindrance.

3D tissue imaging

3D visualization of renal microstructure is primarily limited to confocal microscopy. However, the refractive properties of protein and lipid components within the kidney scatter light and significantly degrade image quality with increasing depth. Optical tissue clearing methods have been employed to address this issue by removing pigments, lipids, and other light-scattering substances from tissues, allowing light to better penetrate the kidney. Combined with deep tissue labeling strategies and the development of optical projection tomography and multiple fluorescence microscopic imaging technologies such as confocal, multiphoton or light sheet, 3D scalable renal studies of cells and molecules have become practicable. Besides traditional tissue clearing methods such as the 3D imaging of solvent-cleared organs (DISCO) family, clear lipid-exchanged acrylamide-hybridized rigid imaging/

Table 1: Promising spatial omics technologies in kidney injury research.

Technology	Classification	FOV	Coverage	Resolution	FFPE-supportive	Used in kidney injury	Reference
Spatial transcriptomics							
10× Visium	Sequencing-based	6.5 × 6.5 mm	Untargeted	55.0 μm	Yes	Yes	[14]
Stereo-seq	Sequencing-based	1 × 1 cm, max 13 × 13 cm	Untargeted	0.2 μm	No	No	[15]
Seq-Scope	Sequencing-based	Max 800 mm^2	Untargeted	0.5 μm	No	No	[16]
Pixel-seq	Sequencing-based	1 × 1 mm, max 10 × 10 mm	Untargeted	1.0 μm	No	No	[17]
HDST	Sequencing-based	5.7 × 2.4 mm	Untargeted	2.0 μm	No	No	[18]
MERFISH	Imaging-based	1 × 1 cm	10,000 genes, proteins	0.2 μm	Yes	No	[19]
10× Xenium	Imaging-based	400 mm^2	400–5,000 (2024) genes, proteins	0.2 μm	Yes	No	[20]
Nanostring CosMx SMI	Imaging-based	100 mm^2	1,000–6,000 (2024) genes, proteins	0.2 μm	Yes	No	[21]
Spatial proteomics							
PCF/CODEX	Imaging-based	2 × 2 cm	>100 proteins	0.5 μm	Yes	Yes	[22]
Spatial metabolomics							
MALDI-MSI	Imaging-based	>7.5 × 2.5 cm	Untargeted, metabolomics, lipidomics, peptides and proteins	5.0–20.0 μm	Yes	Yes	[23]
GCIB-SIMS	Imaging-based	1 × 1 cm	Untargeted, metabolomics, lipidomics, peptides and proteins	1.0 μm	No	No	[24]

Since most omics studies of kidney injury were performed in murine models, technologies with field of view matched with the size of murine kidneys as well as single-cell resolution were displayed. FOV, field of view; PMID, PubMed identifier; FFPE, formalin-fixed paraffin-embedded; HDST, high-definition spatial transcriptomics; MERFISH, multiplexed error-robust fluorescence *in situ* hybridization; SMI, spatial molecular imager; PCF, PhenoCycler-Fusion; CODEX, co-detection by indexing; MALDI-MSI, matrix-assisted laser desorption/ionization mass spectrometry imaging; GCIB-SIMS, gas cluster ion beam secondary ion mass spectrometry.

immunostaining/*in situ* hybridization-compatible tissue-hydrogel (CLARITY) and clear, unobstructed brain/body imaging cocktails and computational analysis (CUBIC), novel strategies like small-micelle-mediated human organ efficient clearing and labeling (SHANEL), raman dye imaging and tissue clearing (RADIANT) and nanobody (V_HH)-boosted 3D imaging of solvent-cleared organs (vDISCO) are developed to be compatible with depth and multiplexity [25]. Now it is feasible to achieve multiplex co-localization with about 30 channels within an intact human kidney or within an entire mouse. Technical compatibility with subsequent pathology and transmission electron microscopy (TEM) allows for integration structural information [26]. By combining molecules indicating segment or cell type with markers of injury-induced heterogenous pathological function, future 3D multiplex renal imaging will be vastly helpful in resolving heterogeneity obstacles caused by segment and spatial location in mechanism studies of kidney injury.

Multimodal integration of imaging and omics

Implementation of multi-modal imaging and omics integration of tissue structure, renal function and molecular information enables the possibility to interpret intricate microenvironments of kidney injury. However, the methodology and technology roadmap for multi-modal integration are spiny and bumpy. With this initiative, an attempt was made and provided us with precious practices of organizing multimodal imaging and omics to achieve a systematic description of nephropathy mechanisms. The Kidney Precision Medicine Project (KPMP) focuses on AKI and CKD, with the goal of establishing a human kidney tissue map with spatial localization at single-cell resolution in both healthy and diseased conditions. KPMP integrated various single-cell sequencing, single-nucleus sequencing,

and spatial imaging technologies such as multiplexed protein imaging and metabolic mass spectrometry imaging in the same renal sample to construct the most comprehensive human kidney map to date [27]. A total of 51 major cell types were identified, and 28 cell states during the process of kidney injury were defined in this research. Distinct spatial localization of protein forms in the vasculature, medulla, cortex regions as well as injury-related cellular neighborhoods and co-expression network within the kidney were sufficiently revealed. Spatial imaging and omics techniques were employed to localize these states within the vicinity of the injury. For example, 3D imaging techniques such as light sheet fluorescence microscopy (LSFM) were utilized to provide information about the immune microenvironment in the injured kidney. This study serves as a paradigm and provides tools for multimodal imaging analysis of the kidney.

Creating a comprehensive, 3D spatially resolved cellular and molecular atlas is required for achieving the final goal of establishing and developing systematic diagnosis and evaluation system of kidney injury. Though not with flawless integration, KPMP is an encouraging attempt of great value in providing us with the first paradigm and many opportunities for reaching this landmark. Indeed, overcoming the above challenges is still highly required for the fulfillment of this goal.

Perspectives

The development of human medicine is largely driven by booming technologies. Recent advances in imaging and spatial omics did provide us with an opportunity to construct an unprecedented, high-resolution, panoramic, dynamical and non-destructive 3D atlas of kidney injury that is spatiotemporally resolved. The field, depth, resolution, scale and throughput of imaging and spatial omics are under active development and have been expanded with more competitive parameters. The higher spatial resolution of *in vivo* functional imaging is gradually approaching that of *in vitro* structural imaging. Compatibility with FFPE samples, and implementation of 3D or high-throughput multi-omics offers endless possibilities of structural and molecular integration. Application of these technologies in kidney injury will finally reach the brand-new landmark and give rise to unprecedented integration of structure, function as well as cellular and molecular mechanisms (Figure 1). A comprehensive evaluation system of kidney disease, as well as a

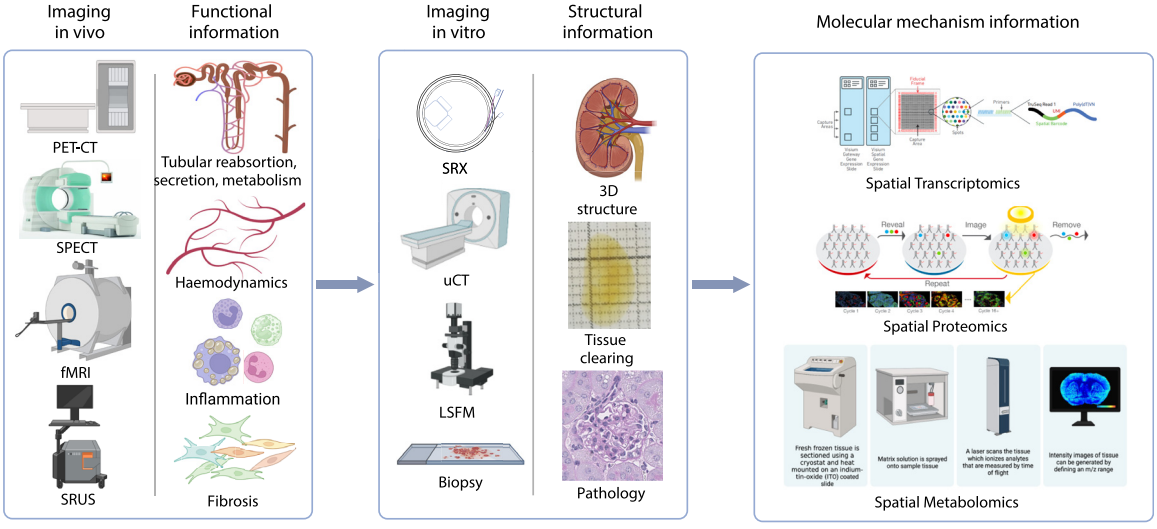

Figure 1: Multimodal integration strategy of renal function, structure and molecular mechanism. Renal physiological or pathophysiological function information such as tubular epithelial function, hemodynamics, inflammation level, fibrosis status are mainly provided by imaging technologies *in vivo* such as positron emission tomography-computed tomography (PET-CT), single-photon emission computed tomography (SPECT), functional magnetic resonance imaging (fMRI) and super-resolution ultrasound (SRUS), thus is given first. After the animals are sacrificed, kidneys are suitable for structural imaging *in vitro* such as synchrotron radiation X-ray (SRX), micro-computed tomography (uCT) or even light sheet fluorescence microscopic (LSFM) imaging after tissue clearing. Biopsy pathology could also provide detailed structural information in cellular level. The kidney slices after structural imaging can also be used to obtain molecular mechanism information by spatial transcriptomics, spatial proteomics and spatial metabolomics. Figures were created with BioRender.com.

systematic interpretation of pathogenesis network of kidney injury will finally be catalyzed.

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