Exploration of renal space: navigating injury and repair through spatial omics

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APPENDIX

English summary
Nederlandse samenvatting
List of publications
Curriculum Vitae
Acknowledgements

ENGLISH SUMMARY

Tissue injury and repair are strongly influenced by cellular metabolism and by interactions between cells within the tissue microenvironment. The kidney is an organ known for its complex tissue architecture, high metabolic demands, and limited capacity to regenerate after injury. Technical advances in so-called spatial omics technologies now allow for mapping of metabolic processes and cellular interactions within the kidney in a spatially resolved manner, directly in the context of the tissue. This thesis focuses on development and application of mass spectrometry imaging (MSI) and data analysis strategies for assessment of the metabolic microenvironment to gain insight into the role of metabolism in the regulation of kidney injury and repair.

Chapter 2 provides an overview of the current state of the field of spatial metabolomics in the context of tissue injury and regeneration. The major advantage of MSI compared to traditional (single cell) metabolomics is that essential information on spatial heterogeneity and tissue architecture is preserved. MSI allows direct detection of metabolites in situ without the need for labeling. The review highlights three key aspects. First, the concept of metabolic molecular histology is discussed, in which metabolite profiles serve as a new layer of histological information capable of revealing known and novel cellular states. Second, the importance of measuring metabolic dynamics is emphasized. Not only the presence but also the metabolic turnover rate provides crucial information about cell function and repair processes. Finally, the integration of spatial metabolomics with other omics disciplines is reviewed. Combining metabolomics with transcriptomics and proteomics has the potential to further unravel metabolic networks and identify new therapeutic targets.

After this conceptual framework, **chapter 3** focuses on the application of MSI to study early metabolic alterations in the diabetic kidney. Diabetes mellitus is one of the leading global risk factors for chronic kidney disease and leads to diabetic nephropathy in nearly half of patients. Current clinical diagnostics largely rely on markers of glomerular function, such as albuminuria, which only appear when structural damage is already present. To intervene before irreversible damage occurs, insight into the earliest metabolic alterations at the cellular level is required. In this chapter, an experimental mouse model of diabetes was used to map kidney metabolic landscapes compared to non-diabetic controls. Metabolite and lipid data were analyzed per nephron segment, with lipid composition serving as a multivariate molecular signature to identify cell types in situ. The most striking alterations occurred in the proximal tubule S3 segment (PT-S3), located in the outer medulla. Specifically, changes were observed in phosphatidylinositol lipid composition, with longer, unsaturated fatty acid chains being replaced by shorter, more saturated ones. These alterations indicate reduced membrane stability and increased injury susceptibility of this segment. Thus, the PT-S3 segment emerges as a previously under-appreciated key player in the early metabolic changes of the diabetic kidney.

Chapter 4 describes the further methodological development toward measuring metabolic dynamics. Traditional MSI analyses have been limited to mapping steady-state metabolite abundances without providing information on the direction or rate of metabolic processes. To overcome this, a method was established for ex vivo stable isotope tracing combined with single-cell level MSI. In this approach, tissue slices are exposed to isotopically labeled nutrients (e.g. ^{13}C-glucose), after which MSI visualizes incorporation of this labeled substrate into downstream metabolites. This enables spatial and single-cell-level visualization of metabolic dynamics and fluxes. The chapter serves as a detailed protocol, outlining critical experimental steps that determine data quality, for example vibratome sectioning to matrix selection. This work describes the foundation for spatial dynamic metabolomics.

In **chapter 5**, this dynamic approach is applied to the context of kidney cancer, specifically renal cell carcinoma (RCC). The main aim of this chapter is to provide a step-by-step outline of the data analysis workflow accompanying spatial dynamic metabolomics in the context of this disease. RCC cells are known for the Warburg effect, a metabolic adaptation in which cells preferentially perform glycolysis over oxidative phosphorylation despite sufficient oxygen availability, resulting in reduced tricarboxylic acid (TCA) cycle flux and high lactate production. Using spatial dynamic metabolomics, tumor cells were compared with healthy proximal tubule cells, from which RCC typically arises, to assess whether this Warburg effect could be demonstrated. The analyses revealed a clear decrease in ^{13}C-glucose-labeled TCA intermediates, indicating diminished mitochondrial activity. Surprisingly, the expected increase in labeled lactate was not observed. Possible explanations for this include negative feedback due to lactate accumulation, or alternative pathways such as histone lactylation, where lactate acts as an epigenetic regulator. Despite this complexity, the study confirmed that RCC cells reprogram their energy metabolism toward glycolysis and demonstrated the power of MSI with stable isotope tracing to visualize such metabolic reprogramming in situ. The chapter serves as a practical guide for understanding and reproducing the complex data analysis required for interpreting spatial dynamic metabolomics datasets.

While chapter 5 focused on metabolic alterations in cancer, **chapter 6** addresses tissue repair after acute kidney injury (AKI). A key determinant in the transition from AKI to chronic kidney disease is the failure of injured proximal tubule cells to properly recover, resulting in so-called failed repair proximal tubule (FR-PT) cells. Previous studies have mainly focused on these cells themselves, but this chapter takes a spatial multi-omics approach to investigate their surrounding niche. By correcting the ionization bias of every endogenous metabolite signal using a stable isotope-labeled internal standard derived from yeast, metabolite abundances were made quantitatively comparable across regions within the tissue. This revealed that seemingly healthy epithelial cells displayed metabolic dysregulation when located in a niche containing FR-PT and interstitial cells. Succinate and linoleate were two metabolites that respectively accumulated or decreased in epithelial cells when situated in an injury context. Corresponding transcriptomic data confirmed disrupted regulation of oxidative phosphorylation and fatty acid metabolism. These findings

indicate that two weeks after AKI, persistent metabolic niches remain within the tissue where cells are metabolically impaired, thereby hampering repair. This calls for a paradigm shift in which not only FR-PT cells but also their surrounding niche should be considered a therapeutic target to prevent progression toward chronic kidney damage.

Together, the chapters of this thesis demonstrate that spatial metabolomics is a powerful tool to chart the complexity of metabolic processes in the kidney and, when integrated with other spatial omics methods, to better understand the role of metabolism in injury and regeneration. The results reveal that metabolism is not merely an intrinsic property of cells but a context-dependent process that spans niches within the tissue. This thesis contributes spatial insights into the role of metabolism in kidney injury and repair, and outlines perspectives on how such knowledge may be leveraged to improve regeneration and prevent chronic kidney failure.