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

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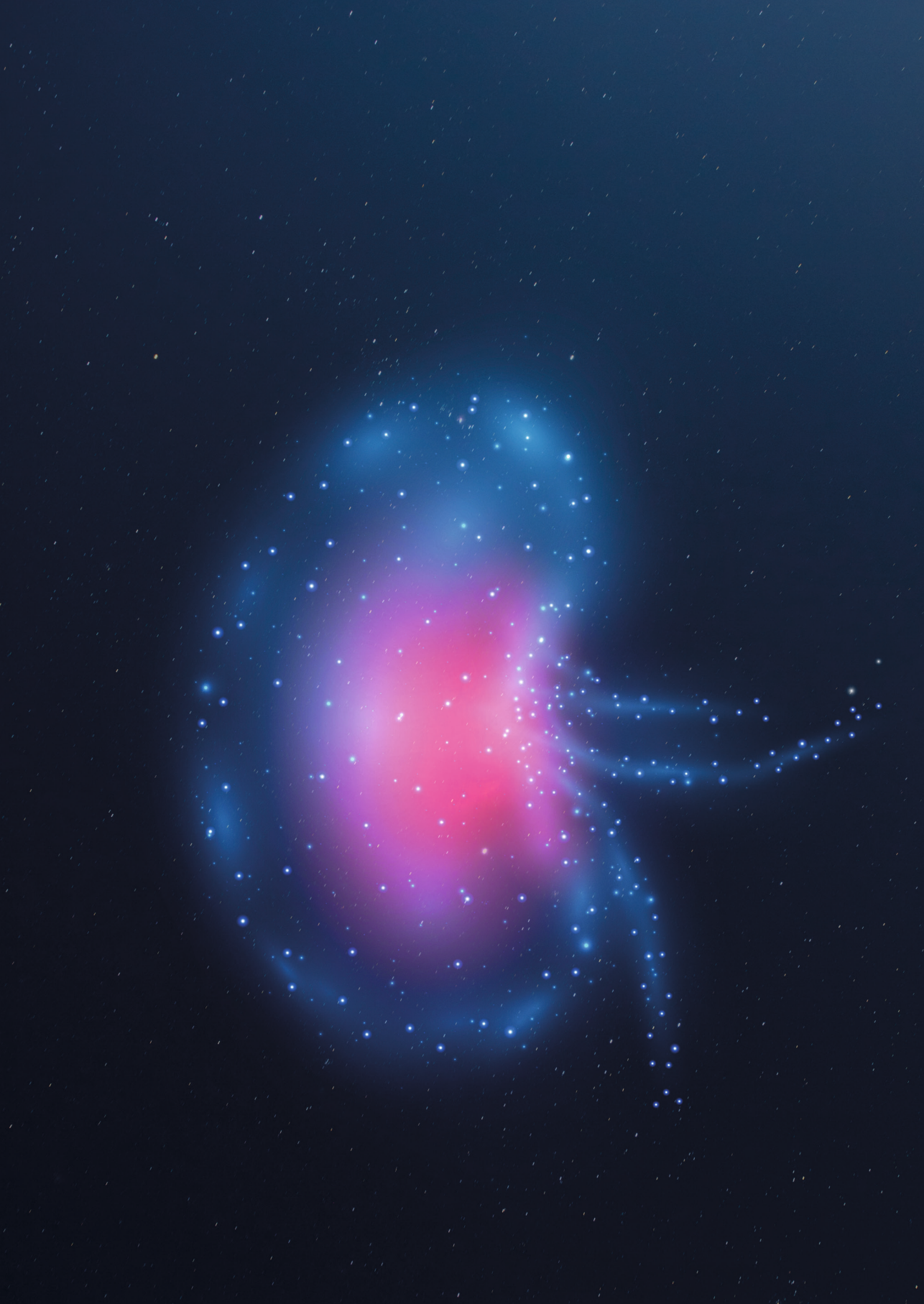

CHAPTER 2

Spatial metabolomics in tissue injury and regeneration

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ABSTRACT

Tissue homeostasis is intricately linked to cellular metabolism and metabolite exchange within the tissue microenvironment. The orchestration of adaptive cellular responses during injury and repair depends critically upon metabolic adaptation. This adaptation, in turn, shapes cell fate decisions required for the restoration of tissue homeostasis. Understanding the nuances of metabolic processes within the tissue context and comprehending the intricate communication between cells is therefore imperative for unraveling the complexity of tissue homeostasis and the processes of injury and repair. In this review, we focus on mass spectrometry imaging (MSI) as an advanced platform with the potential to provide such comprehensive insights into the metabolic instruction governing tissue function. Recent advances in this technology allow to decipher the intricate metabolic networks that determine cellular behavior in the context of tissue resilience, injury, and repair. These insights not only advance our fundamental understanding of tissue biology but also hold implications for therapeutic interventions by targeting metabolic pathways critical for maintaining tissue homeostasis.

INTRODUCTION

Adaptations in cellular metabolism are key within the tissue microenvironment to maintain homeostasis or to initiate a response to injury.[1] Changes in metabolic fluxes determine chromatin accessibility through direct metabolite modifications of histones and determine subsequent downstream transcriptional responses and ultimately cell fate decisions.[2,3] Being informed about the metabolic interaction between cells in a tissue microenvironment is thus relevant to understanding processes such as homeostasis, injury and regeneration. Although conventional (single cell) metabolomics approaches provide extensive cellular metabolic profiles, information of spatial heterogeneity and composition of the tissue microenvironment is lost. We here discuss the current state of the art with respect to spatial metabolomics, an approach that allows *in situ* interrogation of cellular metabolism.

THE RISE OF SPATIAL METABOLOMICS

The field of spatial metabolomics has made rapid advancement in recent years, with mass spectrometry imaging (MSI) being the predominant enabling technology. To date, MSI remains the workhorse in spatial metabolomics studies.[4] Initially, these studies were limited by spatial resolution and molecular class identification, but rapid progression in MSI instrumentation now allows imaging of thousands of molecules like metabolites, lipids, proteins and glycans at a (sub)cellular resolution.[5,6] MSI methods can vary in e.g. ionization technology or method of detection, which is all well described by Reyzer & Caprioli.[7] In this review, we mainly focus on the matrix-assisted laser desorption ionization (MALDI) method, which has seen extensive technological advancement and is the most used technique in biological research settings.[8] In MALDI-MSI, a chemical matrix is applied to a tissue section, thereby extracting analytes from the tissue and forming *in situ* molecule-matrix crystals (Figure 1). These crystals desorb the laser energy, causing the molecules to ionize and making them amenable for measurement by mass spectrometry (MS). As this method does not require any labeling, MALDI-MSI is a powerful tool that enables unlabeled investigation of the metabolic content of biological tissues in a spatial manner.

As for every analytical technique, MALDI-MSI has its limitations and technical challenges.[10] These can be attributed to the sample preparation process (e.g. post-mortem degradation, molecular delocalization or matrix application), MALDI-MSI measurement (e.g. spectral or spatial resolution, ionization bias or in-source fragmentation) and to metabolomics data analysis (e.g. analyte annotation, absolute quantification and multi-omics integration). *Box 1* describes these current challenges and limitations in more detail.

The tissue microenvironment and metabolic molecular histology

The importance of the molecular context in which cells reside is widely recognized in cancer studies, where the tumor microenvironment significantly affects disease progression and

therapeutic outcome.[11,12] Similarly, the tissue microenvironment (TIM) plays a crucial role in tissue regeneration post-injury, with altered metabolite availability and oxygen tension necessitating metabolic adaptations to meet cellular energy demands.[13] Recent studies highlight the pivotal role of metabolite pool alterations in processes beyond metabolic energy transduction, including nutrient sensing and storage, cell survival and differentiation, and immune activation and cytokine secretion.[14-17] Characterization of the TIM using spatial metabolomics is crucial for our fundamental understanding of the role of metabolic processes in tissue homeostasis.

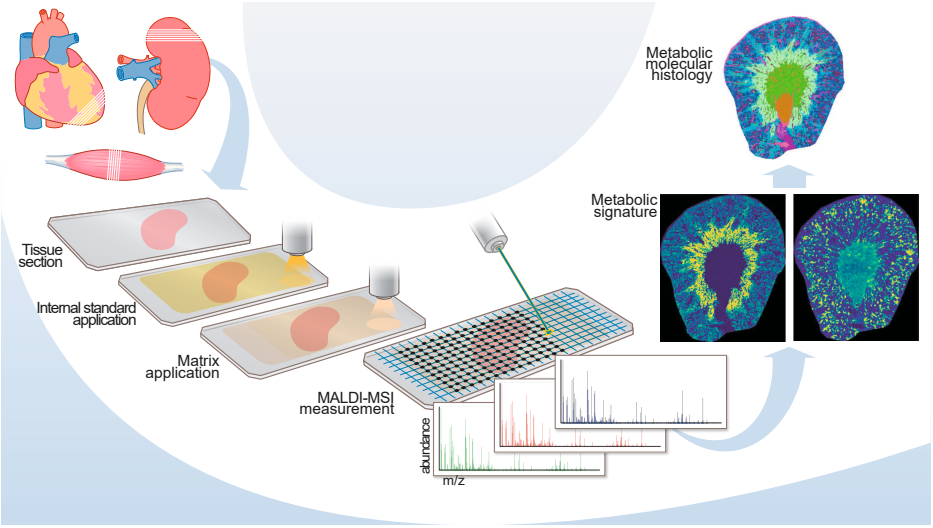

Figure 1: Metabolic molecular histology. Sections of fresh frozen tissues are applied to a slide for the MALDI-MSI pipeline. For qMSI, internal standards are first applied (refer to *Box 1* for details). In all MALDI-MSI experiments, matrix is uniformly applied to the section, facilitating extraction of analytes from the tissue and formation of crystals to make them amenable for mass spectrometry analysis. In the mass spectrometer, spectra are recorded in a spatially correlated manner by imposing a virtual coordinate grid on the tissue. This enables visualization of the analyte distribution within the tissue context. Subsequent dimensionality reduction and segmentation allow for the reconstruction of metabolic molecular histology. The molecular histology images were adapted from previous reports.[9]

The lipid membrane composition has proven to be a powerful tool for dissecting the complex cellular composition of the TIM. Spatial segmentation based on membrane lipids has been used in multiple instances to reveal a so-called metabolic molecular histology (Figure 1).[9,18,19] This histology allows researchers to identify different cell populations and states within these populations, both in health and disease, which are not always revealed with conventional histology approaches.[20] In the kidney, this approach led to the finding of lipid signatures for different renal cells in specific nephron segments and visualized metabolic alterations in cells both preceding tissue injury as well as the altered post-injury TIM.[9,18,19] For the liver, specific hepatic lipid patterns were identified, corresponding to the liver zonation pattern found with immunofluorescent staining.[21] Another study focusing on chronic muscle injury revealed that degenerated muscle regions could be distinguished from

healthy muscle regions based on their lipidomic profile. [22] These examples highlight the strength of spatial lipidomics in its ability to characterize the cellular composition of the TIM.

An additional layer of information that can be added to the molecular metabolic histology is that of spatial proteomics. Imaging mass cytometry and multiplexed ion beam imaging by time of flight (MIBI-TOF) started to pave the way to bring this into practice, however these techniques remain limited due to necessity for highly specialized instrumentation and low throughput. [23,24] Recently, a new MALDI-MSI based spatial multi-omics technique has been developed which combines spatial metabolomics with spatial proteomics using mass-tag-labeled antibodies on post-MSI tissue. This technique, called MALDI-IHC, is based on photocleavable mass-tags that are amenable for measurement with MSI instrumentation, which enables highly multiplexed IHC combined with conventional MSI. [25-27] Addition of proteomics to the molecular metabolic histology allows for a deeper and more comprehensive understanding of the TIM.

Once the composition of the TIM is dissected, the metabolic state of different cell types can be interrogated to decipher the metabolic networks important for tissue homeostasis. As spatial metabolomics allows hundreds of molecules to be measured at the same time, the technique can be applied to look for either metabolic signatures or metabolites that are specific for cell populations or injured sections of the TIM. The strength of the technique is that there is no need for labeling, which makes it applicable to a large variety of tissues and pathologies, which is illustrated by the following examples. A study investigating the early effects of myocardial ischemia revealed that MSI could detect the ischemic cardiac region, whereas this was challenging before due to lack of diagnostic markers. [28] A damage product of nicotinamide adenine dinucleotide (NADH) was one example of a metabolite that was clearly increased in the ischemic region, potentially being a new diagnostic marker. In a second study, MSI was applied in a rat model of ischemic brain injury[29]. In this model, glutamate abundance seemed to be decreased in the infarct area, whereas it was increased in the peri-infarct area. [29] Interestingly, cell therapies tested in this study showed a location-specific effect; information that would be lost with a regular metabolomics strategy. These examples showcase that the identified signature metabolites might provide new therapeutic ways to specifically target the affected TIM upon injury to promote tissue regeneration and homeostasis.

Metabolic dynamics unveiled: MSI meets isotope tracing

Conventional spatial metabolomics studies with MSI have been restricted to capturing metabolite pool-sizes, which allows construction of the metabolic molecular histology but is limited to providing static 'snapshot' of cellular metabolic states. Metabolism is a highly dynamic process, which necessitates a method to elucidate the direction and turnover rate of metabolic pathways. A promising method to achieve *in situ* measurements of metabolic dynamics, is to combine stable isotope tracing with subsequent MSI analysis. [9,30-32] In this approach, a stable isotope-enriched metabolic substrate is introduced in a biological

system, allowing the uptake, turnover and incorporation of this labeled substrate into downstream metabolites to be measured with MSI (Figure 2). For example, U-$^{13}C_6$-glucose as a substrate facilitates tracing of glycolysis, the tricarboxylic acid (TCA) cycle and other metabolic pathways related to glucose metabolism. The most common ways of introducing the stable isotope are through *in vivo* infusion (Figure 2A) or *ex vivo* tissue culture (Figure 2B). The integration of stable isotope tracing with MSI not only expands our understanding of metabolic dynamics, but it also paves the way to reveal metabolic pathways which could be targeted for therapeutic interventions aiding tissue homeostasis (Figure 2C).

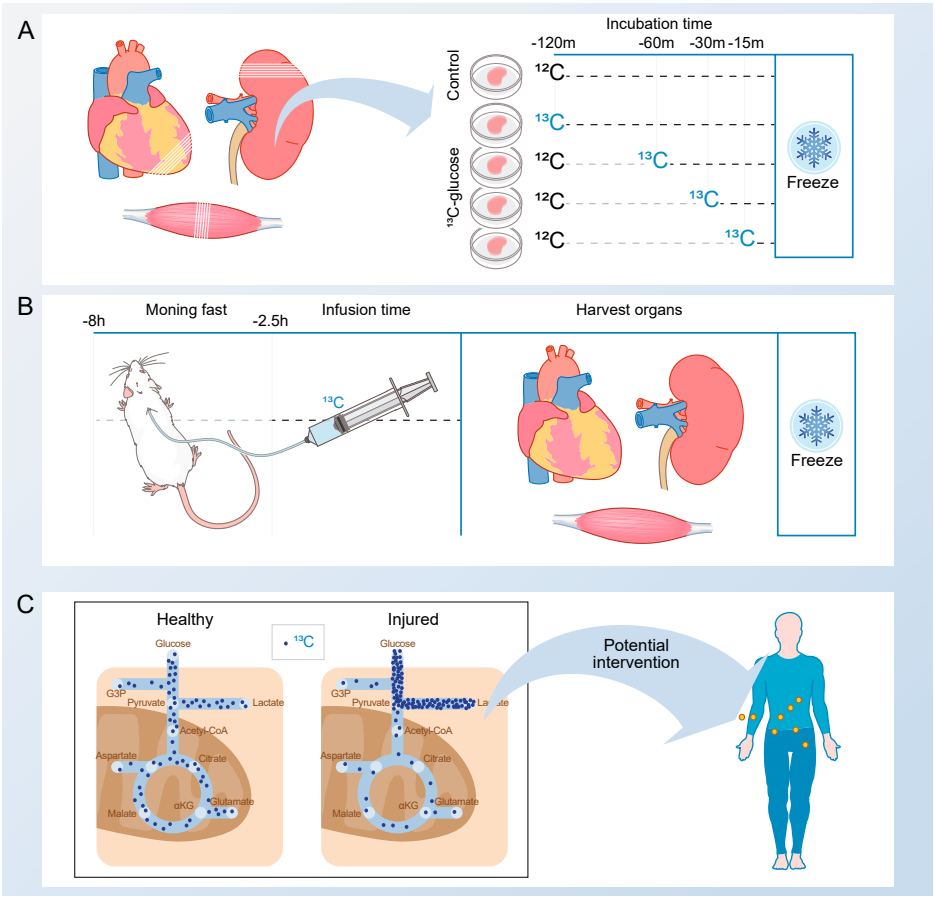

Figure 2: Dynamic metabolic measurements. A: *Ex vivo* tissue slices are incubated at various timepoints with the ^{13}C-enriched stable isotope. After the experiment is done, the sections are frozen down and are subjected to the MALDI-MSI workflow as depicted in *Figure 1*. B: The ^{13}C-enriched nutrient is infused in the animal, after which the organs are harvested and frozen down for subsequent MALDI-MSI analysis as depicted in *Figure 1*. C: Dynamic metabolic calculations can be performed to interrogate the metabolic heterogeneity between healthy and injured tissue. This comparison can reveal potential new therapeutic interventions, promoting tissue regeneration and homeostasis post-injury.

Role of metabolic flux in tissue injury and regeneration

The applicability and biological relevance of dynamic metabolic measurements has been recently exemplified in various publications. [9,30,33] Work by the groups of Davidson and Rabinowitz unveiled regional metabolic preferences in the kidney, highlighting a clear glycolytic and gluconeogenic preference of the medulla and cortex, respectively. [30] By increasing the spatial resolution, Wang *et al*. were able to interrogate metabolic preferences at the single-cell level. [9] This study revealed a higher glycolytic flux and altered ^{13}C-enrichment of TCA cycle intermediates in the maladapted proximal tubule cells after ischemic reperfusion injury compared to their healthy counterparts. Strikingly, unexpected metabolic anomalies were identified in proximal tubule cells with a seemingly normal phenotype in the recovery phase, emphasizing the impact of the TIM. In parallel, spatial dynamic metabolomic measurements were used to improve *in vitro* generation of kidney organoids. [33] By understanding the metabolic trajectory of the developing human kidney, a defect in metabolic maturation of the kidney organoids was identified. Addition of the metabolite butyrate to the culture media resulted in enhanced *in vitro* differentiation and maturation of proximal tubules. Similar approaches applied to the TIM may reveal novel metabolic targets crucial for tissue regeneration after injury.

An exciting, yet challenging frontier in this line of technology involves integrating *in situ* isotope tracing with metabolic flux analysis. In a recent publication, fractional fluxes – the fraction of a metabolite pool which is newly synthesized during the isotope labeling period – were calculated and visualized throughout the brain to investigate the degree of metabolic heterogeneity in the tumor microenvironment compared to surrounding healthy tissue. [31] By adjusting established tools typically used for LC/MS-based metabolomics analysis towards application for MSI, the work of Schwaiger-Haber et al. introduced a workflow that allows fractional fluxes of metabolic pathways to be quantitatively compared between different conditions. Applying this method in a tissue regeneration context allows quantitative evaluation of therapeutic interventions on tissue architecture restoration. Taking it one step further, application of flux modeling algorithms on spatial isotope tracing datasets holds promise for spatially resolved flux measurements during tissue injury and regeneration. A major hurdle in accurate modeling of metabolic fluxes is the need for absolute quantification, which MSI does not provide. Up until now, non-spatial LC/MS-based methods are necessary in parallel to ensure reliable quantification results. Insights derived from such experiments can provide a foundation for developing targeted therapeutic interventions that restore homeostasis and facilitate tissue repair. [34,35]

Spatial metabolomics in the multi-omics era

Similar to other omics technologies, spatial metabolomics generates extensive datasets, containing molecular information from a considerable number of pixels. With spatial resolution improving, the number of pixels per dataset is increasing drastically. Furthermore, the unprocessed datasets contain nonessential information such as adducts or fragments formed during the MALDI process. Spatial metabolomics datasets can reach terabits in size,

underscoring the need for tailored computational methods for effective MSI data processing and analysis. Given the technical artefacts present in the data, like adduct formation and ion suppression, there is no single software program available yet that is able to provide a ready-to-use analysis platform. One approach for handling a MSI dataset is to treat it as a transcriptomics data matrix using pipelines like *Seurat* and *Scanpy*, where each pixel represents a cell and each mass is a feature, which eliminates the need for dedicated image analysis pipelines. [36,37] After processing, the spatial coordinates of pixels can be reintroduced to reconstruct the spatial images. Ongoing efforts are expected to establish more streamlined pipelines to improve MSI data processing in the future.

In the broader omics landscape, integrating data from various modalities has a prominent focus, aiming to construct a comprehensive, multimodal view of cellular states. [38,39] Combining metabolomics with other omics modalities is crucial, as these layers, though distinct, are closely connected in affecting cellular function. Epigenomic analysis can reveal how differences in metabolism influences the chromatin landscape, whereas proteomics (e.g. through MALDI-IHC) can reveal which enzymes might be responsible for different metabolic dynamics. The ability to integrate spatial metabolomics with other omics modalities relies on advancing computational methods. One example is the single cell spatially resolved metabolic (*scSpaMet*) framework, which incorporates MSI with imaging mass cytometry protein maps to enable correlation of features as well as downstream analysis of the joint data. [40] Given the complex, multi-dimensional, ill-understood nature of the multi-omics data, another promising computational approach is to apply deep learning methods which are known for their capacity to identify correlations in this type of data. This application of artificial neural networks offers a promising avenue for extracting new biological insights from complex multimodal datasets.

CONCLUSIONS

Metabolism plays a critical role in determining cellular behavior and function in the process of tissue homeostasis, injury and regeneration. Spatial metabolomics approaches are well suited to study metabolism *in situ*, enabling exploration of intricate metabolic networks within tissue complexity. This review highlighted three key aspects of application of spatial metabolomics. Firstly, the utility of metabolic molecular histology was discussed in revealing cell states. Secondly, the significance was underscored of dynamic metabolic measurements in elucidating altered metabolic processes during tissue injury and regeneration, with implications for refining differentiation strategies and identification of treatment opportunities. Finally, it was explained how the integration of spatial metabolomics with other omics technologies calls for advancements in bioinformatic tools for data interpretation. Insights from this new player in the field of (spatial) omics hold great promise for advancing our fundamental understanding of tissue biology as well as for identifying potential new therapeutic interventions improving tissue regeneration after injury.

Box 1: Limitations and challenges of spatial metabolomics

Spatial metabolomics comes with limitations and challenges, both in experimental considerations as well as data processing and analysis. Given the highly dynamic nature of metabolism, quality of the starting material greatly impacts the resulting quality and resolution of the image. [41] Post-mortem degradation and molecular delocalization need to be prevented as much as possible, by swift tissue handling, freezing and storage, as well as employing properly optimized MALDI matrix application methods. [42] Proper sample handling and preparation methods, combined with matrix crystal size, laser spot diameter and MALDI stage movement together determine the resolution of the resulting MALDI images.

Next, spectral resolution and quality needs to be considered, which is for a significant part determined by the MS instrumentation. Spectral resolution is critical for metabolite annotation, as insufficient resolution may combine multiple molecules into a single peak, and mass accuracy is equally essential for confidently assigning identities to mass features. Quality of the spectra always need to be assessed, to be able to filter out ions that are caused by e.g. in-source fragmentation, adduct formation or natural isotope abundance. Currently, merely empirical and fragmentary knowledge is available about these spectral artefacts, necessitating manual curation of the data. [43]

Finally, a significant hurdle in the spatial metabolomics field is quantification. Quantitative mass spectrometry imaging (qMSI) requires strategies to deal with variations in ionization efficiency between molecules, which can be achieved through use of internal standards. [44-46] Use of internal standards on the one hand allows correction for unwanted signal variability and on the other hand is required for absolute quantification via calibration curves. qMSI has been applied successfully to investigate drug delivery, but also to visualize metabolic fluxes in a complex tissue. [47,48] Furthermore, the possibility of omics-scale qMSI for lipid analysis has been reported using a combination of 13 internal standards for different lipid classes. [49] The challenge remains to apply a similar approach for metabolites, due to the variety in molecular structure.

Recently, a collaborative effort by the MSI field has been done to evaluate various spatial metabolomics protocols and technologies. [50] These efforts, combined with further development of methods will help to establish standard operating procedures, to obtain reproducible and reliable data and to overcome the limitations and challenges of the technique.

REFERENCES AND RECOMMENDED READING

Wang 2022 Nat Metab: This study presented a novel way of studying the metabolic dynamics in the kidney using MALDI-MSI and isotope tracing. The method provided insights into cell type-specific metabolic changes in response to injury.

VandenBosch 2023 Anal Chem: This study demonstrated a quantitative MSI method to analyze various lipid classes *in situ*, enabling comprehensive analysis of lipid distribution.

Wang 2022 Cell Stem Cell: Using a multi-omics platform, this study examined the metabolic trajectories during human kidney development. Insights from this study could be used to improve the *in vitro* differentiation and maturation of hiPSC-derived kidney organoids.

Schwaiger-Haber 2023 Nature Comm: MSI was combined with stable isotope tracing to uncover metabolic alterations in glioma tumors and adjacent healthy tissue. This approach unveiled significant changes in fatty acid metabolism, highlighting the importance of this pathway in glioma.

Lim 2023 Front Chemistry: The MALDI HiPLEX-IHC method was introduced in this study, which enable highly multiplexed, multimodal imaging of tissues at a high spatial resolution.

1 Ghosh-Choudhary, S., Liu, J. & Finkel, T. Metabolic Regulation of Cell Fate and Function. *Trends Cell Biol* 30, 201-212 (2020).

2 Merkuri, F., Rothstein, M. & Simoes-Costa, M. Histone lactylation couples cellular metabolism with developmental gene regulatory networks. *Nat Commun* 15, 90 (2024).

3 Wang, Y. *et al*. The glycolytic enzyme PFKFB3 drives kidney fibrosis through promoting histone lactylation-mediated NF-kappaB family activation. *Kidney Int* (2024).

4 Watrous, J. D., Alexandrov, T. & Dorrestein, P. C. The evolving field of imaging mass spectrometry and its impact on future biological research. *J Mass Spectrom* 46, 209-222 (2011).

5 Pacholski, M. L. & Winograd, N. Imaging with mass spectrometry. *Chem. Rev.* 99, 2977-3006 (1999).

6 Gilmore, I. S., Heiles, S. & Pieterse, C. L. Metabolic Imaging at the Single-Cell Scale: Recent Advances in Mass Spectrometry Imaging. *Annu Rev Anal Chem (Palo Alto Calif)* 12, 201-224 (2019).

7 Reyzer, M. L. & Caprioli, R. M. in *The Encyclopedia of Mass Spectrometry* 285-304 (2016).

8 Zhu, X., Xu, T., Peng, C. & Wu, S. Advances in MALDI Mass Spectrometry Imaging Single Cell and Tissues. *Front Chem* 9, 782432 (2021).

9 Wang, G. *et al*. Analyzing cell-type-specific dynamics of metabolism in kidney repair. *Nat Metab* 4, 1109-1118 (2022).

10 Chaurand, P. in *MALDI Mass Spectrometry Imaging: From Fundamentals to Spatial Omics New Developments in Mass Spectrometry* (ed T. Porta Siegel) Ch. 2, 20-38 (The Royal Society of Chemistry, 2021).

11 Balluff, B., Hanselmann, M. & Heeren, R. M. Mass Spectrometry Imaging for the Investigation of Intratumor Heterogeneity. *Adv Cancer Res* 134, 201-230 (2017).

12 Walsh, L. A. & Quail, D. F. Decoding the tumor microenvironment with spatial technologies. *Nat Immunol* 24, 1982-1993 (2023).

13 Legouis, D. *et al*. Altered proximal tubular cell glucose metabolism during acute kidney injury is associated with mortality. *Nat Metab* 2, 732-743 (2020).

14 Inoki, K., Zhu, T. & Guan, K. L. TSC2 Mediates Cellular Energy Response to Control Cell Growth and Survival. *Cell* 115, 577-590 (2003).

15 Mills, E. L. *et al*. Accumulation of succinate controls activation of adipose tissue thermogenesis. *Nature* 560, 102-106 (2018).

16 Taylor, S. R. *et al*. Dietary fructose improves intestinal cell survival and nutrient absorption. *Nature* 597, 263-267 (2021).

17 Baker, S. A. & Rutter, J. Metabolites as signalling molecules. *Nat Rev Mol Cell Biol* 24, 355-374 (2023).

18 Martin-Saiz, L. *et al*. High-Resolution Human Kidney Molecular Histology by Imaging Mass Spectrometry of Lipids. *Anal Chem* 93, 9364-9372 (2021).

19 Rietjens, R. G. J. *et al*. Phosphatidylinositol metabolism of the renal proximal tubule S3 segment is disturbed in response to diabetes. *Sci Rep* 13, 6261 (2023).

20 Bergman, H. M., Lindfors, L., Palm, F., Kihlberg, J. & Lanekoff, I. Metabolite aberrations in early diabetes detected in rat kidney using mass spectrometry imaging. *Anal Bioanal Chem* 411, 2809-2816 (2019).

21 Seubnooch, P. *et al.* Characterisation of hepatic lipid signature distributed across the liver zonation using mass spectrometry imaging. *JHEP Rep* 5, 100725 (2023).

22 Olie, C. S. *et al*. The metabolic landscape in chronic rotator cuff tear reveals tissue-region-specific signatures. *J Cachexia Sarcopenia Muscle* 13, 532-543 (2022).

23 Giesen, C. *et al.* Highly multiplexed imaging of tumor tissues with subcellular resolution by mass cytometry. *Nat Methods* 11, 417-422 (2014).

24 Keren, L. *et al*. MIBI-TOF: A multiplexed imaging platform relates cellular phenotypes and tissue structure. *Sci. Adv.* 5 (2019).

25 Yagnik, G., Liu, Z., Rothschild, K. J. & Lim, M. J. Highly Multiplexed Immunohistochemical MALDI-MS Imaging of Biomarkers in Tissues. *J Am Soc Mass Spectrom* 32, 977-988 (2021).

26 Lim, M. J. *et al.* MALDI HiPLEX-IHC: multiomic and multimodal imaging of targeted intact proteins in tissues. *Front Chem* 11, 1182404 (2023).

27 Claes, B. S. R. *et al*. MALDI-IHC-Guided In-Depth Spatial Proteomics: Targeted and Untargeted MSI Combined. *Anal Chem* 95, 2329-2338 (2023).

28 Aljakna Khan, A. *et al*. Detecting early myocardial ischemia in rat heart by MALDI imaging mass spectrometry. *Sci Rep* 11, 5135 (2021).

29 Tanaka, E. *et al*. Metabolomic analysis and mass spectrometry imaging after neonatal stroke and cell therapies in mouse brains. *Sci Rep* 10, 21881 (2020).

30 Wang, L. *et al*. Spatially resolved isotope tracing reveals tissue metabolic activity. *Nat Methods* 19, 223-230 (2022).

31 Schwaiger-Haber, M. *et al.* Using mass spectrometry imaging to map fluxes quantitatively in the tumor ecosystem. *Nat Commun* 14, 2876 (2023).

32 Miller, A. *et al.* Spatially resolved metabolomics and isotope tracing reveal dynamic metabolic responses of dentate granule neurons with acute stimulation. *Nat Metab* 5, 1820-1835 (2023).

33 Wang, G. *et al.* Spatial dynamic metabolomics identifies metabolic cell fate trajectories in human kidney differentiation. *Cell Stem Cell* 29, 1580-1593 e1587 (2022).

34 Jang, C., Chen, L. & Rabinowitz, J. D. Metabolomics and Isotope Tracing. *Cell* 173, 822-837 (2018).

35 Hubbard, B. T. *et al*. Q-Flux: A method to assess hepatic mitochondrial succinate dehydrogenase, methylmalonyl-CoA mutase, and glutaminase fluxes in vivo. *Cell Metab* 35, 212-226 e214 (2023).

36 Wolf, F. A., Angerer, P. & Theis, F. J. SCANPY: large-scale single-cell gene expression data analysis. *Genome Biol* 19, 15 (2018).

37 Stuart, T. *et al*. Comprehensive Integration of Single-Cell Data. *Cell* 177, 1888-1902 e1821 (2019).

38 Hao, Y. *et al.* Integrated analysis of multimodal single-cell data. *Cell* 184, 3573-3587 e3529 (2021).

39 Bredikhin, D., Kats, I. & Stegle, O. MUON: multimodal omics analysis framework. *Genome Biol* 23, 42 (2022).

40 Hu, T. *et al.* Single-cell spatial metabolomics with cell-type specific protein profiling for tissue systems biology. *Nat Commun* 14, 8260 (2023).

41 Wang, J. *et al*. MALDI-TOF MS imaging of metabolites with a N-(1-naphthyl) ethylenediamine dihydrochloride matrix and its application to colorectal cancer liver metastasis. *Anal Chem* 87, 422-430 (2015).

42 Cillero-Pastor, B. & Cuypers, E. in *MALDI Mass Spectrometry Imaging: From Fundamentals to Spatial Omics New Developments in Mass Spectrometry* (ed T. Porta Siegel) Ch. 5, (Royal Society of Chemistry, 2021).

43 Alexandrov, T. Spatial Metabolomics and Imaging Mass Spectrometry in the Age of Artificial Intelligence. *Annu Rev Biomed Data Sci* 3, 61-87 (2020).

44 Porta, T., Lesur, A., Varesio, E. & Hopfgartner, G. Quantification in MALDI-MS imaging: what can we learn from MALDI-selected reaction monitoring and what can we expect for imaging? *Anal Bioanal Chem* 407, 2177-2187 (2015).

45 Tobias, F. & Hummon, A. B. Considerations for MALDI-Based Quantitative Mass Spectrometry Imaging Studies. *J Proteome Res* 19, 3620-3630 (2020).

46 Taylor, P. J. Matrix effects: the Achilles heel of quantitative high-performance liquid chromatography-electrospray-tandem mass spectrometry. *Clin Biochem* 38, 328-334 (2005).

47 Chumbley, C. W. *et al*. Absolute Quantitative MALDI Imaging Mass Spectrometry: A Case of Rifampicin in Liver Tissues. *Anal Chem* 88, 2392-2398 (2016).

48 Abu Sammour, D. *et al*. Quantitative Mass Spectrometry Imaging Reveals Mutation Status-independent Lack of Imatinib in Liver Metastases of Gastrointestinal Stromal Tumors. *Sci Rep* 9, 10698 (2019).

49 Vandenbosch, M. *et al*. Toward Omics-Scale Quantitative Mass Spectrometry Imaging of Lipids in Brain Tissue Using a Multiclass Internal Standard Mixture. *Anal Chem* 95, 18719-18730 (2023).

50 Saharuka, V. *et al*. Large-Scale Evaluation of Spatial Metabolomics Protocols and Technologies. *bioRxiv* (2024).