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Making Minds Matter

Multi-omic Spatial Mapping of Myocardial Infarction and Implications for Personalized Therapy

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Abbreviations: single cell RNA sequencing (scRNA-seq), single nucleus RNA sequencing (snRNA-seq), single cell assay for transposase-accessible chromatin sequencing (scATAC-seq), single nucleus assay for transposase-accessible chromatin sequencing (snATAC-seq), cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq), transcription factor (TF), Deep Visual Proteomics (DVP), Co-detection by Indexing (CODEX), fluorescence in situ hybridization (FISH), SpaTial Enhanced REsolution Omics-Sequencing (Stereo-seq), whole exome sequencing (WES), chromatin immunoprecipitation sequencing (ChIP-seq), Nanodroplet Processing in One pot for Trace Samples (nanoPOTS), Single Cell ProtEomics by Mass Spectrometry (SCoPE-MS), single-cell energetic metabolism by profiling translation inhibition (SCENITH)

Keywords: spatial transcriptomics, single cell sequencing, multi-omic, myocardial infarction, heart failure, cardiac fibrosis, personalized therapy, targeted therapy

Abstract

Ischemic heart disease including myocardial infarction (MI) is still the leading cause of death worldwide. While the survival early after MI has been significantly improved by the introduction of percutaneous coronary intervention, long-term morbidity and mortality remains high. The elevated long-term mortality is mainly driven by cardiac remodeling processes triggering ischemic heart failure and electric instability. Despite the new developments in pharmaco-therapy of heart failure, we still lack targeted therapies for cardiac remodeling and fibrosis. Single cell and genomic technologies allow to map the human heart at unprecedented resolution and allow to gain insights into cellular and molecular heterogeneity. However, these technologies rely on digested tissue and isolated cells or nuclei and thus lack spatial information. Spatial information is critical to understand tissue homeostasis and disease and can be utilized to identify disease driving cell-populations and mechanisms including cellular cross-talk. Here we discuss recent advances in single cell and spatial genomic technologies that give insights into cellular and molecular mechanisms of cardiac remodeling after injury and can be utilized to identify novel therapeutic targets and pave the way towards new therapies in heart failure.

Introduction

Ischemic heart disease is still a leading cause of mortality worldwide. While most patients survive acute myocardial infarction today [1,2], heart failure development late after MI remains unacceptably elevated, increasing disease burden and mortality.[3–5] Current therapeutic strategies delay heart failure progression but are limited in their ability to halt or reverse fibrosis and adverse cardiac remodeling. Moreover, the clinical translation of therapeutic strategies targeting signaling pathways with widespread function in inflammation and fibrosis has not been successful.[6] Broad anti-inflammatory interventions such as corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) were utilized to reduce inflammation and thought to thereby improve cardiac function after MI.[6] However, these strategies did not improve adverse remodeling after MI but had deleterious effects and led to increased complications.[6] Selective anti-inflammatory interventions showed more promising results, but still target various cell-types across organs thereby potentially resulting in various side-effects. In different trials interleukin 1β (IL1β) inhibition was shown to reduce adverse remodeling after

MI and during heart failure.[6] However, unfortunately, IL1β inhibition also showed an increased susceptibility for fatal infection or sepsis.[6] The failure of these different anti-inflammatory strategies might be in part attributed to high heterogeneity across patients with different genetic background, different comorbidities, age, gender, etc., but also cellular, molecular and spatiotemporal heterogeneity after MI. This highlights the need for novel biomarkers and precision medicine tools for personalized risk stratification and treatment allocation to future clinical trials but also novel high resolution techniques to identify highly specific therapeutic targets.[7] Therefore a better understanding of molecular mechanisms and disease subcategories is essential. The healing after MI and the following adverse remodeling underlie complex molecular mechanisms with various cell types, subtypes and states involved. Recent single cell genomics studies characterized the cell type and molecular heterogeneity in more detail and further identified novel cell states.[8,9] These studies led to a better understanding of molecular mechanisms after MI and identified several novel potential therapeutic targets. But the heterogeneity is not only limited to cell states or signaling pathways. The healing after MI is also highly heterogeneous in a spatio-temporal context. The molecular mechanisms highly differ between different areas within the heart over time. Roughly, the heart can be divided in 3 zones after MI: the infarction zone, the border zone and the remote zone. Each zone is characterized by different cell types, cell-cell interactions, epigenome, transcriptome, proteome, metabolome, etc. changing over time after MI. The pathways and cells involved in different phases after MI significantly differ. In the early inflammatory phase phagocytic cells and inflammatory cytokines dominate to clear the infarcted area from dead cells and debris.[10] Later, the proliferative phase is dominated by reparative macrophages that orchestrate the scar formation and neovascularization and secret growth factors to support fibroblast and endothelial cells in these tasks.[10] Recent advancements in epigenomics, transcriptomics, proteomics and bioinformatics have the potential to significantly improve our understanding of molecular mechanisms in diseases by combining different layers of omics (genomics, epigenomics, transcriptomic, proteomics, metabolomics) to so-called multi-omics datasets. Adding spatio-temporal information to these datasets even increases their potential. This spatial information helps to better understand the local tissue niches that comprise the cellular microenvironment and cell-cell interactions, as well as the localization of cells within the 3-dimensional tissue architecture with gene and protein-expression depending on this spatial context and thus help to more precisely define molecular mechanisms of cardiac injury and remodeling. Spatial transcriptomics are becoming increasingly popular and are now commercially widely available for the broad scientific community. Spatial transcriptomics enable the quantification of mRNAs across the transcriptome within intact tissue sections. Whereas the generation and analysis of one dimensional-omic data sets is well-established for most of the modalities, optimal multi-omics integration and analysis often remains challenging. We are here giving an overview on spatial multi-omics and recent studies and highlighting their potential for target and biomarker discovery.

Single-cell and multi-omics in cardiovascular research

In recent years the improvement of omics technologies tremendously improved our understanding of mechanisms and cells involved in healing after MI. Continuously improving computational methods for the analysis of large datasets enabled researchers around the world to discover disease and cell type specific gene regulatory networks from scRNA-seq or new potential biomarkers from proteomics data.[8,9,11] New computational methods can even

estimate cell-cell interactions [12], receptor-ligand interactions [13], pathway activity or cell differentiation trajectories[14] from scRNA-seq data. However, it is critical to mention that these computational tools only build models that need further validation. Further, all computational tools have their pro´s and con´s. It is thus recommended to test several tools in parallel. Receptor-ligand interaction tools that give a broad overview around potential crosstalk signaling pathways such as CellphoneDB for example lack information on whether there is indeed binding of the ligand to the receptor and downstream target gene activation.[15]

Powerful computational approaches are crucial to unleash the full potential of multi-omics datasets and interdisciplinary cooperation between wet lab scientists and bioinformaticians is generally required for advanced analysis. The field of computational tools is highly dynamic and developing very fast. There are a multitude of powerful approaches for the integration of multi-omics datasets such as iOmicsPASS[16], MOFA[17], sciCAN[18], totalVI[19], COSMOS[20] and various more. There are a multitude of useful computational tools for single cell analyses. The website scrna-tools.org provides a nice overview of currently over 1300 tools.

Several studies have identified new potential targets in an unbiased way and thereby paved the way for further mechanistic and therapeutic studies of cardiac disease. The number of single cell omics studies tremendously increased in the last years. To exemplary show their tremendous potential for target discovery we will briefly discuss some studies combining different single cell multi-omics approaches in MI.

Ruiz-Villalba et al. performed single cell RNA sequencing (scRNA-seq) and chromatin accessibility from assay for transposase accessible chromatin sequencing (ATAC-seq) from murine Collagen1α1–GFP-positive cardiac fibroblasts after MI.[21] They used their dataset to identify new fibroblast subtypes after MI, gene regulatory networks and potential markers of these subtypes. Thereby they identified a novel CD200+/CD146− fibroblasts subtype with reparative and pro-fibrotic properties. The ATAC-seq analysis revealed increased activity of transcription factors such as SRY-Box Transcription Factor 9 (SOX9) potentially critical in the regulation of pro-fibrotic gene expression in reparative fibroblasts after MI.[21] Differential gene expression analysis identified Collagen Triple Helix Repeat Containing 1 (Cthrc1) as a regulator of reparative fibroblasts and to be specifically expressed in fibroblasts. Further validation experiments confirmed the involvement of Cthrc1 in fibrosis, but Cthrc1 knockout in mice led to increased mortality through cardiac rupture after MI which could potentially be due to reduced fibrosis with subsequent aneurysm and left ventricular rupture.[21]

Alexanian et al. recently demonstrated in an elegant study the value of a combination of controlled temporary perturbation of gene-expression and single cell genomics (scRNA-seq, scATAC-seq) for target discovery.[22] In a mouse model of heart failure they discovered the transcription factor mesenchyme homeobox 1 (MEOX1) as regulator of fibroblast activation. The authors induced a transitory perturbation in mice after transverse aortic constriction (TAC) surgery using a bromodomain and extra-terminal motif (BET) inhibitor JQ1 which abrogates cardiac fibrosis. The combination of scRNA-seq and scATAC-seq enabled the authors to dissect the gene regulatory networks and transcription factor activities involved in pro-fibrotic fibroblast subtype differentiation. MEOX1 was found among the transcription factors with increased activity in activated fibroblast.[22] Further validation experiments could identify the cis-regulatory regions controlling *Meox1* expression and decreased fibrosis after knockdown of *Meox1 in vitro*.[22]

Li et al. combined an endothelial `Confetti´ lineage tracing mouse combined with single cell transcriptomics to unravel the cellular and molecular mechanism governing neovascularization after MI.[23] Beside clonal expansion of resident endothelial cells and novel

subtypes based on transcriptomic data, the authors discovered plasmalemma vesicle-associated protein (PLVAP) as a potential novel target to enhance neovascularization after MI.[23] *In vitro* knockdown experiments confirmed the pro-angiogenic effects of PLVAP on endothelial cells.[23]

Nowadays, non-transcriptomic based single cell omics are still complicated to perform and not well established. Further development of single cell proteomics, metabolomics, phosphoproteomics etc. is still urgently needed and will probably bring tremendous new findings to the field. However, combining techniques such as proteomics with single cell transcriptomics has already led to significant findings. Proteomics are much better suited for the discovery of novel biomarkers. Chan et al. recently demonstrated that the combination of serum proteomics and scRNA-seq supports the prioritizing of novel biomarker candidates before further investigations.[24] The authors measured 1305 serum proteins from patients one month post-MI and analyzed them for differentially expressed proteins associated with subsequent heart failure and cardiac function. They further prioritized the differentially expressed proteins using scRNA-seq data from mice and humans. The top prioritized proteins included known biomarkers such as N-terminal B-type natriuretic peptide and troponin T, but also new ones such as angiopoietin-2 (ANGPT2), thrombospondin-2 (THBS2), latent transforming growth factor-β binding protein-4 (LTBP4), and follistatin-related protein-3 (FSTL3) that need to be validated in further studies.[24]

Vafadarnejad et al. used an approach combining cellular indexing of transcriptomes and epitomes by sequencing (CITE-seq) and scRNA-seq to dissect the temporal heterogeneity of cardiac and blood neutrophils after MI.[25] In their study the authors identified a tissue-specific cell state with increased expression of Sialic acid-binding Ig-like lectin F (SiglecF) and increased phagocytic activity.[25]

Recently, Chaffin et al.[26] and Reichart et al.[27] published some of the largest snRNA-seq datasets of human cardiomyopathy (dilatative, arrhythmogenic and hypertrophic) up to now (600,000 and 881,081 nuclei respectively). Through their unprecedented resolution, these datasets allowed the recovery of rare cell types and subtypes such as neuronal cells, mast cells, lymphatic endothelial cells, etc. This further allowed the discovery of novel cell states (e.g. novel fibroblast states)[27] and potential therapeutic targets in activated fibroblasts such as extracellular matrix protein prolargin (*Prelp*) and juxtaposed with another zinc finger protein 1 (*Jazf1*).[26] Therefore, they are an important source for the field and will pave the way for a better understanding of the molecular and cellular mechanisms in heart failure and for the development of novel targeted therapies.

In summary, these studies demonstrate the tremendous potential of single cell multi-omics for the discovery of novel potential targets for personalized medicine. Multi-omics can predict cell specificity and help to identify the most promising candidates, thereby accelerating the discovery of novel targets. However, these techniques still lack spatial resolution which is crucial to reliably understand cell-cell interactions, cell microenvironment as well as cellular and molecular spatial differences of the infarcted heart.

Spatial omics in cardiovascular research

In recent years single cell transcriptomic studies have identified several new cell types and states. However, we still lack a precise understanding where these cells are exactly located in space in complex tissues such as the heart. Are they located in the infarction area, in the border zone or remote zone? Which cell types are involved in different fibrosis types (interstitial

fibrosis, perivascular fibrosis, replacement fibrosis)? We need spatial context in our data to better dissect mechanisms that drive remodeling in different areas of the heart. For example, after MI we need to limit adverse remodeling whereas we shouldn´t inhibit scar formation which would lead to increased mortality through failed fibrosis and left ventricular rupture. Which myofibroblast or fibroblast cell type and state drives which process and which cells can be targeted without risking aneurysm formation and rupture? Spatial context will help us to answer these questions and thus affect how to choose new targets and implications for new therapies.

Spatial omics are only emerging in the last few years. Currently the field is mainly dominated by spatial transcriptomics which was recently highlighted as the method of the year 2020 by *Nature Methods*.[28] Advances in multiplexed imaging technologies and mass spectrometry (MS) technologies have drastically improved our ability to characterize spatial distribution of proteins in tissues and single cells.[29–32] Different MS-based (e.g. Deep Visual Proteomics (DVP))[29] or imaging based (e.g. Co-detection by Indexing (CODEX))[32] technologies enable us nowadays to assess spatial distribution of several hundreds to thousands of proteins in a single sample. Other spatial technologies need to be further improved to become accessible to the broad scientific community. However, first findings using spatial epigenomics[33] or metabolomics[34] in other organs were published recently and give hope that these technologies might become accessible to the broad scientific community soon. These technologies will certainly improve our understanding of spatial differences in heart after MI and also during heart failure development from other causes including heart failure with preserved ejection fraction (HFpEF).

Spatial transcriptomics can profile cellular microenvironments and individual cells while retaining spatial information.[35] This is essential for the understanding on how cellular neighborhoods and disease pathology influence gene expression, cell states, cell abundance and to monitor the molecular interactions between tissue components.[35] Over the last decade spatial transcriptomics have evolved rapidly in sensitivity, multiplexing and throughput. They can be divided into two different technologies: sequencing-based technologies and imaging-based technologies.[35–37] Sequencing-based spatial transcriptomics use DNA-barcoded slides which capture mRNA locally from intact tissue and mark the mRNA from distinct spatial spots using specific barcodes. The barcoded mRNA can then be transcribed to cDNA, amplified and sequenced using next-generation sequencing.[35,38] Imaging-based spatial transcriptomics use microscope techniques to simultaneously image several mRNA transcripts within a tissue. They use either in situ sequencing[39,40] or fluorescence in situ hybridization (FISH).[35,41,42] Sequencing-based technologies are limited in resolution to their spot-size which recover several cells in one spot (50-200µm diameter). Decreasing the spot size or bead-size (e.g. SlideSeq[43]) usually results in a decreased detection of mRNA. However, there is tremendous development in this field and various technologies can increase the resolution while retaining a high detection of gene expression. For example, SpaTial Enhanced REsolution Omics-Sequencing (Stereo-seq) achieves large field-of-view spatial transcriptomics at cellular resolution by combining DNA nanoball (DNB) patterned arrays and tissue RNA capture.[44,45] The Stereo-seq technology allows an unprecedented resolution and sensitivity in the field of sequencing-based technologies. [44]

Imaging-based spatial sequencing can have up to a subcellular resolution. The downside of imaging-based technologies is that they are limited to a lower number of genes that can be targeted and usually have to be pre-selected in a panel. Sequencing-based technologies theoretically recover mRNA from all expressed genes but are biased towards highly abundant mRNAs.[35] Depending on the scientific question, researchers need to choose the appropriate

technique or combine them. Concerning cardiovascular research, only studies using sequencing-based spatial transcriptomics investigating MI have been published yet.[46–52]

Recent findings using spatial multi-omics approaches

So far spatial multi-omics studies investigating MI mainly focused on single cell transcriptomics, epigenomics, proteomics and spatial transcriptomics. Studies adding other modalities are missing, but will certainly emerge in the next few years to give more precious spatial insights into the healing and remodeling after MI. Here we will present the currently available studies and their main findings to illustrate the potential of spatial multi-omics for a better understanding of cell-cell interaction, cell abundance, distribution of cell subtypes and states in space, cell microenvironment and further target discovery.
Early this year, Ko et al. used scRNA-seq, spatial transcriptomics and proteomics to better understand the interaction between cardiomyocytes and fibroblasts in post-MI heart failure development.[47] Through single-cell co-expression network analysis, they identified high-temperature requirement A serine peptidase 3 (Htra3) in cardiac fibroblasts to be a critical regulator of cardiomyocyte homeostasis and regulator of cardiac fibrosis. Htra3 showed up to be important for cardiac homeostasis and TGFβ degradation. Using a Htra3 knockout mouse in models of murine heart failure (TAC) and myocardial infarction confirmed a detrimental effect on fibrosis and heart failure in knockout mice due to abnormal fibroblast to cardiomyocyte crosstalk.[47] Spatial transcriptomics revealed an increased expression of Tgfβ and insulin-like growth factor-binding protein 7 (Igfbp7), a mediator of cell senescence [53], in the infarction area of knockout mice. Serum-Proteomics and scRNA-seq from human samples showed an increased expression of Igfbp7 in failing cardiomyocytes as well as an increased serum concentration of IGFBP7 in patients with advanced heart failure. This suggested that IGFBP7 might be a promising biomarker in post-MI heart failure, whereas targeting Htra3 might improve cardiac function in heart failure.
Jung et al. used scRNA-seq from $CD45^+$ leukocytes and spatial transcriptomics in a time course after murine MI to identify the heterogeneity and spatiotemporal dynamics of leukocytes at a single-cell level after MI.[48] Besides other leukocyte subtypes, this approach led to the identification of a macrophage subtype ($Trem2^{hi}$ macrophages) with increased expression of triggering receptor expressed on myeloid cells 2 (Trem2) late after MI. Further validation showed an anti-inflammatory and reparative phenotype of $Trem2^{hi}$ macrophages with important roles of these macrophages during healing after MI.[48] Trem2 injection into the infarction area significantly improved the cardiac function and healing after MI, suggesting that targeting $Trem2^{hi}$ macrophages might be an interesting target to support healing after MI.
Recently, two studies integrated scRNA-seq and spatial transcriptomics of various time points after MI to dissect the cellular and molecular mechanisms driving remodeling in the border zone after MI.[51,52,54] Both studies highlighted the critical role of mechano-sensing genes for the remodeling in the border zone early after MI. Calcagno et al. discovered two layers of cardiomyocytes with distinct gene expression and specific spatial localization (border zone 1 (BZ1) is adjacent to the remote zone, whereas border zone 2 (BZ2) is adjacent to the ischemic zone).[52] The authors identified increased expression of genes associated with mechano-sensing, focal adhesion and mechano-transduction in cardiomyocytes of the border zone. Therefore, they proposed a “loss of neighbor” hypothesis whereby surviving cardiomyocytes signal the loss of stability they experience to their neighbors. Interestingly, the loss of neighboring cardiomyocytes *in vitro* (scratch assay) increased the expression of the same mechano-sensing genes in cardiomyocytes, supporting the “loss of neighbor” hypothesis.[52] In

accordance with these findings Yamada et al. observed increased gene expression of mechano-sensing genes in the border zone. They identified the mechano-sensing gene Cysteine and glycine-rich protein 3 (*Csrp3*) as a regulator of post-MI remodeling in the border zone after MI. Gene silencing and overexpression of *Csrp3* demonstrated that upregulation of *Csrp3* regulates genes associated with mechano-sensing and thereby prevents cardiac remodeling after MI.[51] These studies dissected the spatial gene expression in the border zone in an unprecedented resolution. The technical difficulties to microdissect the border zone limited previous studies that did not use spatial transcriptomics.

Newer studies used human tissue after MI for the generation of spatial multi-omics maps. We recently published the most substantial spatial multi-omics data set from human myocardial infarction so far.[46] We combined and integrated single nucleus RNA-seq (snRNA-seq), single nucleus ATAC-seq (snATAC-seq) and spatial transcriptomics to generate a spatial multi-omics map of human myocardial infarction.[46] This led to the identification of different cell niches (fibrotic, inflammatory, myogenic), dependencies between fibroblast and myeloid cells, different functional states of cardiomyocytes and endothelial cells depending on their location in space (injured vs. uninjured cardiomyocytes), the influence of tissue microenvironment on cell states and function. The data showed a unique spatial interaction between myofibroblasts and activated phagocytic macrophages (Spp1^{+} CD36^{+}). The identified interactions between cell types reflect the spatial organization of the tissue and provide various hypotheses for further studies.

Finally, Amrute et al. recently published a preprint investigating the immune-fibrosis axis in MI and heart failure.[50] The authors utilized CITE-seq, snRNA-seq and spatial transcriptomics in human hearts from non-diseased donors, chronic ischemic and non-ischemic cardiomyopathy patients. The authors dissected fibroblast cell states after MI and in chronic heart failure and identified a fibroblast trajectory marked by fibroblast activation protein (*Fap*) and periostin (*Postn*) expression that is modulated by macrophages and inflammatory cytokines and contributed to cardiac fibrosis. Targeting these inflammatory cytokines abrogated the emergence of Fap^{+} fibroblasts and reduced cardiac fibrosis. Further, targeting Il1β in a murine model of cardiac injury showed a differentiation trajectory away from activated Postn^{+} fibroblasts and towards the myofibroblast lineage. The Il1β targeting therapy improved cardiac function and abrogated adverse remodeling, suggesting that the Fap^{+} fibroblast lineage might be a promising target in cardiac fibrosis.

Several of the discussed studies identified different fibroblast subtypes and highlighted subtypes involved in fibrosis.[8,9,11,21,22,26,27,46,50] However, the heterogeneous nomenclature of fibroblast subtypes and different marker genes used in recent sc/snRNA-seq and multi-omics studies make it difficult to draw reliable conclusions on which fibroblast subtypes are responsible for adverse remodeling in cardiac fibrosis. Therefore, standardization of the annotation of subtypes is urgently needed and current summaries should be seen as preliminary and used with caution. However, we still tried to summarize the current data generated by scRNA-seq and multi-omics on fibroblast subtypes involved in adverse remodeling and cardiac fibrosis: Recent studies suggest that myofibroblasts per se might not be the sole source of extracellular matrix (ECM) in cardiac fibrosis. Previous studies rather support the idea that another fibroblast subtype might also be involved in adverse remodeling and cardiac fibrosis. Most studies named this subtype activated fibroblasts, but other terms such as ECM-fibroblasts exist.[9,11,21,26,46,50] This subtype is characterized by marker genes such as periostin (*Postn*), fibroblast activator protein (*Fap*), Collagen triple helix repeat-containing protein 1 (*Cthr1*), Mesenchyme Homeobox 1 (*Meox1*), Thrombospondin 4 (*Thbs4*), but does not express classical contractile marker genes of myofibroblasts such as alpha smooth muscle

actin (*Acta2*).[9,11,21,26,46,50]. Myofibroblasts are essential for the mechanical stability after MI and for a proper scar formation, however, different studies investigating fibrosis in non-ischemic heart failure identified activated fibroblasts (or ECM-fibroblasts) as main source of ECM and did not recover myofibroblasts in their data.[11,26] Since myofibroblasts are essential for healing after MI and new data highlights the role of activated fibroblasts in cardiac fibrosis, the latter might be a more promising target in cardiac fibrosis. Interestingly, Aghajanian et al. demonstrated that the ablation of these activated fibroblast using FAP targeting Chimeric Antigen Receptor T-cells (FAP CAR-T cells) significantly reduces cardiac fibrosis, supporting the hypothesis that these activated fibroblasts might be the subtype driving fibrosis.[55] This raises the question on how we define myofibroblasts. Historically myofibroblasts have been termed activated fibroblasts and the addition "myo-" was added to reflect the expression of contractile fibers. An alternative terminology defines myofibroblast as the cells that produce most matrix[56]. In our opinion it would be important to agree on definitions and names for the identified mesenchymal cell populations including fibroblast subtypes and myofibroblasts and also other cell-states (e.g. endothelial, cardiomyocytes). Integration of most available datasets with a common annotation of cell types and states would be of major importance for the field.
In summary, the first studies using a spatial multi-omics approach improved our understanding of cell-cell interactions, cell microenvironment and cell abundances and states in different regions of the heart after MI. This led to the discovery of potential new targets and biomarkers for the treatment of MI and post-MI remodeling.

Implications of spatial multi-omics approaches for personalized medicine and targeted therapy

Recent studies demonstrate that spatial multi-omics have high potential to improve our understanding of molecular mechanisms after MI and thereby unravel new potential diagnostic and therapeutic targets. When performed at single cell resolution (e.g., scRNA-seq) these technologies can additionally predict the cell type specificity and help to choose targets with probably limited adverse side effects. The spatial context helps to understand cell microenvironment and to locate different targets to specific regions after MI (e.g., remote zone, border zone, infarction zone). The spatial information can help to target specific niches, cell-cell interactions but also specific cardiac regions. As an example, to reduce adverse remodeling, we need to abrogate fibrosis in the remote and border zone, whereas it should not interfere with scar formation in the infarction area since this might lead to increased mortality by triggering aneurysm formation and potential left ventricular rupture. One strategy to avoid this would be a therapeutic regime that starts after the reparative process of the central MI scar is finalized.
Previous studies showed that therapeutic stimulation of angiogenesis is associated with reduced scarring and improved outcomes in animal models of acute MI.[57] A deeper understanding of post-MI angiogenesis will help to improve therapeutic strategies. To further promote neo-angiogenesis within infarcted areas after MI, the understanding of molecular mechanisms in the border zone might be more useful than the analysis of vasculature in the remote myocardium. Spatial multi-omics might also help here to identify the areas of interest e.g. border-zone and thus study neovascularization in a zone-dependent fashion to understand mechanisms and find new targets. The ultimate goal is to find new biomarkers to classify patients in subgroups according to their risk and either allocate subgroups to available re-purposed therapies or to develop novel targeted therapies that might differ for each subgroup. Spatial multi-omics data sets have the potential to unravel new potential biomarker

and therapeutic targets that can be used for further validation studies. The data sets and also technologies are quickly advancing, and current limitations are certainly the resolution or depth and the cell-annotation since sequencing-based approaches usually detect gene expression in a given spot that contains genes of several cells even if the spot-size is smaller than an average cell. In imaging-based approaches cell borders can be stained by antibodies to be able to distinguish which RNA is expressed in which cell. However, these technologies have the same limitation as conventional imaging-based approaches of tissue where cells that are closely located and e.g., macrophages wrap around fibroblasts or myofibroblasts cannot be distinguished particularly since the tissue section has a z-projection and cytoplasm of one cell might lie under the cytoplasm of a second cell. However, computational and wet lab technologies are quickly being developed that allow improved annotation and demarcation of cell types within these approaches.

Future perspectives

While first spatial multi-omics studies have given us unprecedented insights into cell microenvironment and spatial heterogeneity after MI, most of the work still has to be done. Emerging spatial multi-omics technologies are currently mainly based on transcriptomics. Spatial and single cell epigenomics, proteomics, metabolomics, etc. are more challenging and not well established yet. More advances are needed here to make these technologies available to the broad scientific community. These are needed to further improve our understanding of molecular mechanisms in healing after MI. We cannot understand a complex organism using only one layer. Each layer has advantages. For example, proteomics are better suited to identify new biomarkers and increased mRNA expression does not necessarily mean increased protein amount while signaling pathways are often regulated by phosphorylation.

Spatial transcriptomics are still either limited by their resolution, throughput or number of genes that can be analyzed. Further improvements in resolution might help to demarcate cell types and dissect cellular cross-talk with subcellular resolution giving novel insights of intracellular dynamics and repartition of RNA transcripts and protein expression.

However, to develop new personalized therapies we will also need a better understanding of patient subgroups. Different patient subgroups might benefit from different targeted therapies. Possibly, age, gender, genetics, comorbidities and various other factors might influence the healing after MI and adverse remodeling in a way that different therapeutic strategies might be needed. To achieve the best outcome for each individual patient we therefore need specific new biomarkers to classify these patients and determine risk for adverse remodeling and patient-specific interventional approaches. Further studies are needed here to not solely investigate MI in general but rather investigating disease pathways and mechanisms for different patient subgroups (e.g., age, gender, comorbidities such as diabetes, chronic kidney disease, clonal hematopoiesis, etc.). The integration of phenomics into multi-omics data might also help to further improve diagnostics and risk stratification. Phenomics use large data sets of clinical data and computational tools or machine learning for the systematic study of disease specific traits (phenotype) to identify novel markers for diagnostics or predictors of disease progression.[58] The integration of phenomics into spatial multi-omics data sets could help to identify specific gene-regulatory programs in patient subgroups with different phenotype (e.g. different cardiac function or adverse remodeling). Thereby, the molecular and cellular mechanisms that lead to more adverse remodeling or worse cardiac function could be identified. This could help to define optimal treatment strategies for subgroups of patients.

While single cell epigenomics are often integrated in multi-omics datasets (e.g. scATAC-seq, scChIP-seq, scDNase-seq)[59], single cell post-genomics have, to the best of our knowledge, not been integrated yet into multi-omics datasets (with exception of CITE-seq). The emergence of single cell post-genomics and potential integration with multi-omics will be another exciting field to follow in the next few years. These emerging technologies will allow us to get a more complete picture of the cellular and molecular mechanisms in tissue homeostasis and disease. Several new technologies emerged in the last years for the investigation of single cell metabolomics and proteomics. Single cell metabolomics can be divided in 3 different approaches: i.) mass spectrometry (MS) and MS-imaging based approaches (e.g. SpaceM[60]), ii.) histochemistry-based approaches[61] and iii.) cytometry-based approaches (e.g. SCENITH[62]). Single cell proteomics can roughly be divided in two categories: antibody-based approaches and MS-based approaches. Up to 50 proteins can be detected by antibody staining and consecutive flow cytometry, mass cytometry or high resolution microscopy detection.[63] Sequencing-based approaches using barcoded antibodies such as CITE-seq can detect over 250 proteins per cell, but are limited to surface proteins. [64]. However, these technologies are limited by the amount of detectable epitopes and specific antibody panels. CITE-seq only detects surface proteins. Additionally, specific high affinity antibodies do not exist for all proteins. Therefore, the community rather turned to MS-based approaches for non-targeted identification and quantification of molecules.[63] The sample preparation is essential for MS-based approaches due to the limited amount of proteins in single cells. Different approaches optimized the workflows in the last years (SCoPE-MS[65], nanoPOTS[66]) and will hopefully become accessible to the scientific community soon.
The emerging field of spatial multi-omics technologies is advancing quickly and can help to dissect mechanisms of tissue injury and repair at unprecedented resolutions thereby paving the way towards discovery of novel biomarkers and therapeutic targets for a personalized therapy after MI. The number of replicates or patient samples are currently limited and often patient specific differences might mask disease specific effects particularly at early stages of disease. However, in the future novel techniques will likely allow higher throughput at increased resolution and decreased costs. These advances will empower biological discovery and have the potential to answer questions concerning cell composition, cell-cell interaction, and molecular interaction in individual patients.

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Title	Species	omics used	doi	main findings
Spatial multi-omic map of human myocardial infarction	human	snRNA-seq snATAC-seq spatial-seq	10.1038/s41586-022-05060-x	Spatial multi-omic map that identifies different cell niches, cell states and cell interactions in cardiac tissue after MI.
Cardiac fibroblasts regulate the development of heart failure via Htra3-TGF-β-IGFBP7 axis	mouse	scRNA-seq spatial-seq proteomics	10.1038/s41467-022-30630-y	Fibroblast-cardiomyocyte interaction through a Htra3-TGF-β-IGFBP7 axis in failing hearts. Identification of IGFBP7 as a potential novel target in heart failure.
Spatiotemporal dynamics of macrophage heterogeneity and a potential function of Trem2hi macrophages in infarcted hearts	mouse	scRNA-seq spatial-seq	10.1038/s41467-022-32284-2	Identification of Trem2hi macrophage subpopulation that improves healing after MI.
Full-length spatial transcriptomics reveals the unexplored isoform diversity of the myocardium post-MI	mouse	spatial-seq	10.3389/fgene.2022.912572	Identification of spatial isoform diversity in cardiac tissue after MI.
Single-cell and spatial transcriptomics of the infarcted heart define the dynamic onset of the border zone in response to mechanical destabilization	mouse	sc/snRNA-seq spatial-seq	10.1038/s44161-022-00160-3	Dissection of the cellular and molecular mechanisms of the border zone after MI and identification of 2 zones with distinct gene expression.
Spatiotemporal transcriptome analysis reveals critical roles for mechano-sensing genes at the border zone in remodeling after myocardial infarction	mouse	snRNA-seq spatial-seq	10.1038/s44161-022-00140-7	Dissection of the spatiotemporal molecular changes in the border zone after MI and identification of mechano-sensing genes involved cardiac remodeling such as *Csrp3*.
Targeting the immune-fibrosis axis in myocardial infarction and heart failure (preprint)	human	CITE-seq snRNAseq spatial-seq	10.1101/2022.10.17.512579	Characterization of an inflammatory-fibrosis axis and dissection of the myeloid-fibroblast crosstalk.

Table 1: Overview on currently available spatial-seq and spatial multi-omics data sets investigating MI.

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Highlights Section

- Spatial multi-omics are powerful approaches to perform unbiased analysis of the cellular and molecular processes in normal and diseased tissues.
- The spatial information of spatial multi-omics enables a better understanding of local tissue niches that includes the cellular microenvironment, cell-cell interactions, the localization of cells within the 3-dimensional tissue architecture and spatial gene and protein expression.
- Recent multi-omics studies identified several potential new targets for targeted therapy and biomarker for diagnostics.
- Spatial multi-omics studies pave the way for personalized medicine.

Figure 1. From spatial multi-omics to personalized medicine. The analysis of spatial multi-omics data sets is a powerful tool to discover new disease pathways, patient subgroups, biomarkers for diagnosis and prediction as well as potential therapeutic targets for targeted therapy. Beside the improvement of technologies investigating different omics, novel computational methods are crucial to properly analyze the data. The development of specific biomarkers and therapeutics for patient subgroups after MI is a key for personalized medicine with highly effective drugs and reduced adverse side effects. (The illustration was created using BioRender).