Computational strategies in cardiometabolic diseases

Chapter 8

Impact

Scientific Impact

In the past decade, high-throughput technologies are booming and have propelled biomedical research to a new level. The development of single-cell omics (e.g., scRNA-Seq and CyTOF) has enabled researchers to uncover rare cell types and detect cellular heterogeneity. This has made it a valuable tool not only for studying cardiometabolic diseases, but also in other disease domains such as cancer, immunology, microbiology, and neurology [1, 2]. The pathologies of these diseases are typically complex and closely related to the microenvironment of the cells in the diseased tissue. However, as all information of the cell's location is lost due to tissue dissociation steps before analysis, these single-cell technologies are unable to analyse the microenvironment of cells. Some spatial omics techniques, such as 10x Visium[3] (transcriptomics), are unable to identify the phenotypes of individual cells due to their low spatial resolution. In addition, some imaging techniques either do not capture enough features (e.g., immunofluorescent microscopy (IF)) or are expensive (e.g., CODEX [4]). Therefore, biologists urgently require affordable and effective ways to identify cell types while visualizing their spatial distributions and dissecting the cellular and molecular microenvironments. In response to this pressing requirement, I proposed a novel computational pipeline in **chapter 2**. The pipeline implements several functions. First, it allows biologists to distinguish cell phenotypes in an interactive manner based on the relative intensities of multiplex signals in multispectral imaging. Secondly, it visualizes individual cells and cell communities on histological images, helping pathologists to quickly verify their identities. Thirdly, this approach links multiple omics imaging data (i.e., multispectral imaging and MSI) with the corresponding histological images in tissue, allowing to dissect the molecular environment of cells from multiviews. As this approach is affordable and applicable to a wide range of cell types and tissues, it may represent a breakthrough in linking molecular context to cellular phenotype and function in healthy and diseased tissues. In the end, this not only provides new insights into pathogenesis of disease and leads for prevention and/or treatment of cardiovascular disease, cancer, neurological disorders, and a range of other diseases, the recently developed user-friendly interface also brings MSI/multispectral image analysis within the reach of biologists and pathologists, who are not or marginally skilled in R or Matlab.

In the field of spatial metabolomics, MALDI-MSI detects metabolite levels in tissue while preserving spatial information [5]. However, to study metabolite differences across different sections of the tissue, researchers always manually designate the regions of interest (ROIs) on the histological image for each section, and then align the histological image with the optical image from MALDI-MSI. This requires a significant amount of time and effort. Moreover, this approach is error-prone especially if tissue sections used for histology and MS imaging differ (for instance

due to the laser-inflicted tissue damage). In **chapter 3**, I presented a new strategy to identify liver compartments using a combination of supervised and unsupervised MSI segmentation algorithms, with circumvents the need of a histological image. Accurate segmentation of parenchyma, sinusoid, and vessel on MALDI-MSI images indicates the presence of compartment-specific metabolites that are not associated with disease progression. Validation for these identified metabolites is expected to completely free researchers from the time-consuming and repetitive annotation work and to also bring breakthroughs in future studies of metabolism in liver.

In **chapter 4** and **5**, I interrogated a monocyte transcriptomics dataset to extract sex-specific cytokine signalling pathways and hypertension-associated gene networks, respectively. Although both findings still need validation in an independent genetic or genomics dataset, they could pave the way for the design of new drugs for tailored intervention in ischemic heart disease or hypertension-associated heart failure. Moreover, as monocyte isolation is only mildly invasive, the leads could represent new genetic diagnostics for early stages of heart failure and microcirculatory dysfunction, or for the effectiveness of sex-specific interventions in CVD development in women.

Societal Impact

Therapeutic intervention in cardiometabolic diseases is complicated by the profound heterogeneity of disease-driving inflammatory cells. However, this heterogeneity was hitherto only poorly studied. The comprehensive pipeline proposed in **chapter 2** allows to identify an unprecedented number of myeloid phenotypes in murine atherosclerotic tissues and to dissect the cellular and molecular microenvironment associated with these phenotypes. With this approach we are able to demonstrate that plaque myeloid phenotypes often have a unique and characteristic cellular and molecular environment, which offers the possibility of treating atherosclerosis by altering the macrophage microenvironment. Such new insights could eventually benefit many patients as they allow the design of more tailored precision medicines that target the right subset in the right patient.

Non-alcoholic fatty liver disease (NAFLD) is a heterogeneous and complex disease that affects approximately 20% to 25% of Europeans and 30% to 40% of Americans[6, 7]. Metabolic disturbances are one of the main features of NAFLD progression [8]. Therefore, determining the spatial distribution of metabolites on liver tissue and the metabolic changes with disease progression is essential to decipher the heterogeneity of liver tissue and to gain insight into NAFLD. **In chapter 3**, our sptio-temporal analysis of mouse liver tissue and enrichment analysis for metabolic clusters revealed that metabolite clusters within sinusoids and parenchyma during the early stage of NAFLD involve mostly identical and a small

number of differential pathways. While our studies are basic in nature, findings may benefit the design of a new generation of precision medicines or life-style interventions that target the aberrant metabolism in NAFLD. Moreover, the identified disease-associated metabolites could serve as early reporters of the disease.

Substantial evidence has shown that male and female CVD patients differ in terms of underlying causes, presentation, and prognosis of the disease. Meanwhile, most studies on CVD risk factors still mainly focus on male patients [9]. In **chapter 4**, we observed distinct CVD-specific sex differences in monocyte transcriptional profiles and cytokine activities, and female patients are more activated in EGF, IFN1, CD40L, GM-CSF and VEGF signalling pathways than males. More relevant, we Identified a common regulator of the disturbed signalling pathways that may serve as target for genetic linkage studies or for interventions specifically in women. Translating these findings into practice will contribute to reducing gender disparities in preventive care and improving clinical CVD treatments for women.

Hypertension is widely acknowledged as major risk factor for cardiovascular disease and blood pressure control has become one of the main means to prevent cardiovascular diseases [10, 11]. Nevertheless, the underlying mechanism behind the link between them is not clear. In **chapter 5**, we uncovered the negative correlation between blood pressure and LPS response of monocytes, indicating the reason why hypertension is a risk factor for CVD may be related to the suppressed immune response. This finding could improve treatment options for CVD disease associated with hypertension in the future. Furthermore, we inferred that iloprost, a drug capable of targeting this disease network, may not only enhance a compromised LPS response in monocytes in CVD-susceptible subjects, but may also represent an effective drug in the treatment of diastolic hypertension-related CVD.

Conclusion

The computational approaches developed in this thesis greatly facilitate the work of biomedical researchers. Additionally, the findings based on these computational analyses deepen the understanding of cardiometabolic diseases and contribute to the development of more effective therapeutic options for an early conquest of this disease.

References

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