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CARDIOVASCULAR RESEARCH Onlife

Lessons from the spatiotemporal expression patterns of RNA vs. proteins during the cell cycle

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Commentary on 'Spatiotemporal dissection of the cell cycle with single-cell proteogenomics' by Mahdessian *et al.*, *Nature* 2021;590:649–654.

The cell cycle is a fundamental process for the maintenance of life, in which cells organize, grow in mass, integrate growth signals, and finally split into daughter cells. This process is meticulously orchestrated through checkpoints, in which dysfunction can lead to cell death or cell cycle reprogramming,[1] eventually causing proliferative diseases such as cancer. A vast number of proteins are cell-cycle-dependent, and thus their expression varies within phases of the cell cycle.

Mahdessian *et al.*[2] have recently characterized the spatiotemporal expression patterns of RNA and proteins during the cell cycle in unsynchronized and genetically identical human cells. They reported around 20% variability in protein expression between single (individual) cells in time and space within the cell cycle, from those proteins mapped in the Human Protein Atlas. Of 2193 proteins with cell-to-cell variability identified, Mahdessian *et al.*[2] selected 1180 proteins and analysed the correlation with interphase progression by targeted single-cell proteogenomics in Human Bone Osteosarcoma Epithelial Cells (U2OS Line). Interestingly, more than 70% of the proteins analysed (from 1180 in total) presented intercellular variations that were not explained by cell cycle progression. Also, there was only a small overlap between cell-cycle-dependent proteins and their corresponding transcripts. From 320 cell-cycle-dependent proteins detected in single cells, 104 presented a pseudotemporal position fluctuation in their expression pattern in interphase, undetectable when analysed in batches. This is the case, for instance, with NFAT5, which stimulates the expression of plasminogen activator inhibitor-1, promoting fibrin deposition and infiltration of macrophages in endothelial cells.[3] However, SCIN, a regulatory protein involved in exocytosis, presented a high intercellular variability that did not correlate with the cell cycle. The biological and functional consequences of the cell-to-cell proteome variation in genetically identical cells, such as proliferative capacity, drug resistance, or alterations in cell metabolism, needs to be further investigated.

In fact, understanding the molecular changes from single cells in ageing and disease may determine the efficacy of therapeutic interventions. These challenges have recently been addressed by the LifeTime Initiative (https://lifetime-initiative.eu, last accessed in May the 19th 2021), a European Consortium with the objective of implementing cell-based interceptive medicine over the next decade.[4] As depicted in *Figure 1*, single-cell multi-omics analyses are highly relevant for cardiovascular research, from the identification of novel insights into cell-to-cell communication, regulatory networks, and novel developmental or disease mechanisms, but also to possibly influence the redefinition of complex clinical entities, biomarker and drug discovery and drug repurposing, as extensively reviewed.[5] The consortium will contribute datasets to the Human Cell Atlas and Human Protein Atlas initiatives. Understanding the peculiarities of cells from the cardiovascular system may help in the understanding of the disease, as well as in the understanding of the differences between patients.

In a recent paper, Zhao *et al.*[6] analysed the cellular heterogeneity and response to elastase-induced abdominal aortic aneurysm in infrarenal abdominal aortas from C57BL/6J mice by single-cell RNA sequencing. The authors identified four subsets of smooth muscle cells and five subsets of monocytes/macrophages with distinct transcriptional profiles, even in healthy aortas. During the elastase-induced abdominal aortic aneurysm progression, the proportion and transcriptional signature of both smooth muscle cells and monocytes/macrophages subsets varied, indicating that the specific transcriptomic signature of each cell subset may determine its contractile functionality and capacity of inflammation resolution and vascular repair, and consequently, that cell heterogeneity is relevant and may predict the severity of abdominal aortic aneurysm progression.

In another recent manuscript, single-cell sequencing analyses have been performed to elucidate the phenotype of different immune cell subsets expressing CD31 in patients with heart failure.[7] Abplanalp *et al.*[7] showed that around 22–40% of genes are differentially regulated in a cell type-specific manner, pinpointing the differential role of cell subsets in heart failure. In fact, they observed a profound alteration in transcriptional profiles within classical, intermediate, and non-classical monocytes in these patients, and specifically in those genes related to inflammation and anti-angiogenic coding proteins.

Fernandez *et al.*[8] performed a single-cell immune mapping of human atherosclerotic plaques that revealed new innate and adaptive immune

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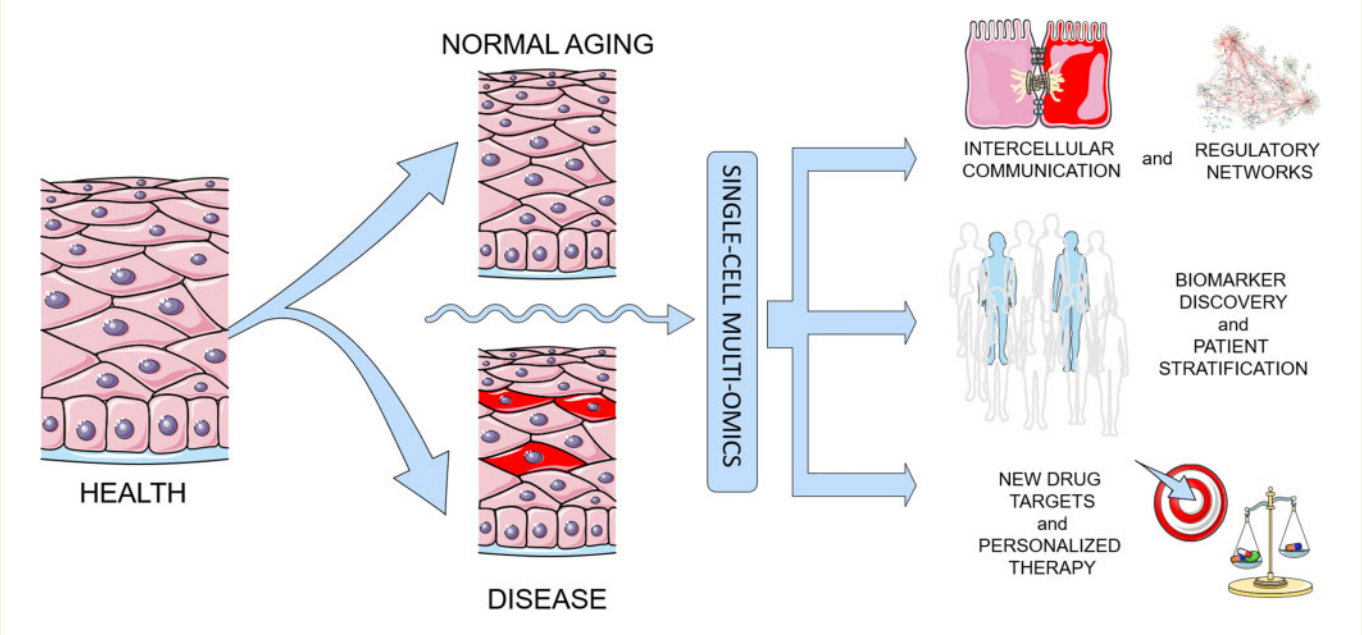

Figure 1. Outline of the single-cell and omics technologies to investigate the cardiovascular system, from health to disease. This figure was created using Smart-Servier Medical Art (https://smart.servier.com, last accessed May the 17th 2021).

dysregulation in atherosclerotic lesions associated with cerebrovascular events, through the combination of mass-cytometry (CyTOF), Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE-seq), and scRNA-seq analyses. They identified 15 subsets of macrophages specific from atherosclerotic tissues with distinct transcriptional signatures. They also identified two specific subsets of $CD8^+$ and $CD4^+$ T cells, respectively, which were only present in symptomatic and not in asymptomatic atherosclerotic plaques. Interestingly, this heterogeneity in T cells was independent from the clinical characteristics of the patients and the plaque pathological classification. In mice expressing *Jak2*^{VF}, a mutation associated with increased risk of atherothrombosis, single-cell RNA sequencing of atherosclerotic plaques revealed an enrichment in inflammatory and resident macrophages subsets compared to plaques from *Jak2*^{VF}*Gsdmd*$^{-/-}$ mice,[9] which correlated with atherosclerotic severity, again directing the attention to the importance of cell singularities in cardiovascular outcomes.

In developmental research, Mantri et al.[10] combined single-cell and spatial transcriptomics to create a hierarchical map of cell lineages and to study the interaction between cell morphogenesis and differentiation in a chicken model of cardiac development. The authors reported four development stages, and a strong correlation within the same developmental stage in spatial and single-cell transcriptomes, thus resolving single-cell space- and time-specific transcriptomic signatures in the cardiac tissue, which identified anatomical regions with singular transcriptional programmes during the four development stages.

While the use of single-cell transcriptomics techniques is rapidly increasing in cardiovascular research, Mahdessian *et al.*[2] have shown that proteomic variations between genetically identical individual cells are largely unexplained by the cell cycle and that temporal dynamics of proteomic cycling is largely regulated translationally or post-translationally. Thus, there will be the need of proteomics in addition to transcriptomics studies at the single-cell level. However, single-cell proteomics is still not an 'off the shelf' technology, because of technical challenges in achieving sufficient proteome coverage and time/cost-effectiveness. Nevertheless, the combination of single-cell omics analyses opens up new perspectives in the study of protein variations in time and space within cell subsets, and their implications in cell and tissue functionality. This information will undoubtedly lead to a deeper knowledge about key regulators of cell cycle and cellular proliferation, which will bring to light new potential diagnostic, prognostic, or therapeutic targets in angiogenesis and cardiomyocyte regeneration.

In conclusion, the study of single-cell spatiotemporal expression patterns of RNA can lead to enhanced knowledge of key regulators of the cell cycle and cellular proliferation, but it will require further investigation at the protein level to harness potential diagnostic, prognostic, or therapeutic targets. Such novel treatments are needed for cardiovascular precision medicine, i.e. to induce angiogenesis, prevent ischaemia/reperfusion injury and stimulate cardiomyocyte regeneration in heart failure.

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Biography: Dr Gemma Chiva-Blanch obtained a PhD in Medicine from the University of Barcelona, Spain, in 2013, where she investigated the effects of diet in atherosclerosis. She obtained two postdoctoral competitive grants, to work at the ICCC in Barcelona, to study the potential of extracellular vesicles as biomarkers of cardiovascular disease. She performed a research stage at the Oslo University Hospital, Norway, deepening into the relationship between extracellular vesicles, thrombosis, and myocardial infarction. She is currently a CIBEROBN Research Fellow at the IDIBAPS in Barcelona, Spain, investigating on the role of extracellular vesicles in atherothrombosis, diabetes, and obesity. She is a member of the ESC Working Group on Thrombosis (2016), where she became a Nucleolus member of the Young Thrombosis Group (YTRG) in 2017 (2017–2020), being the liaison between the YTRG and the Scientists of Tomorrow. This year, she has become a member of the ESC Scientists of Tomorrow Nucleus.

Biography: Dr Manuel Mayr qualified in Medicine from the University of Innsbruck (Austria) in 1999. He then moved to London to undertake a PhD. Upon completion of his PhD, he achieved promotion to Professor in 2011. He has been awarded a prestigious British Heart Foundation Personal Chair in 2017. His academic achievements have been recognized by the inaugural Michael Davies Early Career Award of the British Cardiovascular Society (2007), the inaugural Bernard and Joan Marshall Research Excellence Prize of the British Society for Cardiovascular Research (2010), and the Outstanding Achievement Award by the European Society of Cardiology Council for Basic Cardiovascular Science (2013).