Disease-associated cell states in atherosclerosis defined by spatial transcriptomics

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Background

Atherosclerosis is a complex disease that includes several cell types, most notably macrophages, smooth muscle cells (SMCs) and endothelial cells (ECs)[1]. Spatial transcriptomics provides the possibility to detail the changes in gene expression in tissue context, therefore allowing us to map the localization of different cell types and their disease-associated cell states. Here, we used the Resolve Bioscience Molecular Cartography spatial transcriptomics platform (Figure 1) to capture the spatial locations of individual mRNA transcripts in the arteries of mice under a high-fat diet (HFD). The flexible output data of Molecular Cartography allowed us to perform several image- and bioinformatics-based analyses that clarify the spatial distribution of disease-associated genes and cell states, as well as the gene expression changes and molecular processes behind them.

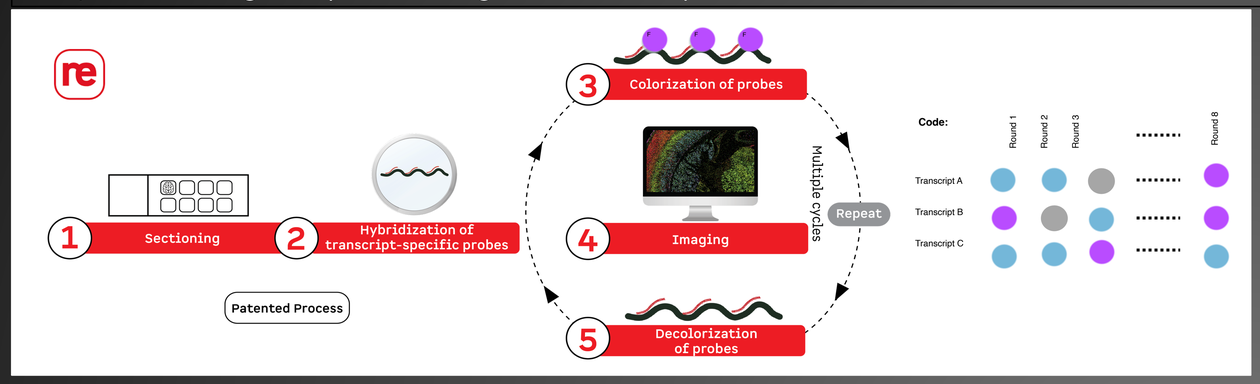

Figure 1: The Resolve Biosciences Molecular Cartography platform. The sample sections are placed on a coverslip, after which the transcript-specific probes are hybridized. Several cycles of probe colorization, imaging and decolorization are performed, which creates a unique colorization code for each transcript.

Results

Leiden Clustering

Performing Leiden clustering using the bioinformatics tool ScanPy[2] we discovered 33 cluters in total, of which the top 10 are presented here (Figure 2). The clustering suggests that the lesion lipid core consists mainly of two separate macrophage cell states, dedifferentiated SMCs and chondrocytes (Figure 2). In the media there is a layer of contractile SMCs present that turn into transitioning SMCs when moving towards the lesion lipid core (Figure 2). A very distinct endothelial layer can also be seen lining the top of the lesion (Figure 2).

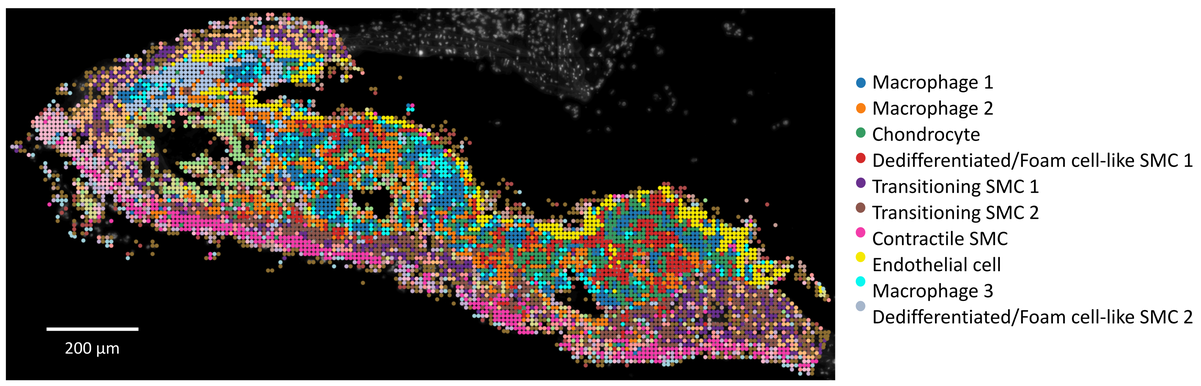

Figure 2: Leiden clustering of atherosclerotic lesion located in mouse aortic root after a 3-month high-fat diet. The top 10 clusters were manually annotated based on top marker genes and are presented on the right side. Each dot has a diameter of approximately 8 µm. Leiden clustering was performed using ScanPy, the figure was created using STLearn.

Cell state marker gene expression

We used the bioinformatics tool STLearn[3] to map the marker gene expression of several disease-associated cell states. What's interesting is the way different cell types and states are distributed within the lesion: macrophages and dedifferentiated SMCs seem to be the main cell states in the lipid core, and individual cell states form separate islands in the lesion samples taken from mice after a 1-month HFD, as is indicated in the expression of the cell state marker genes across the lesion (Figure 3). As the disease progresses, we can see a more uniform distribution of common markers of macrophages (CD68)[4] and dedifferentiated SMCs (*CD44*)[5] within the lesion, but also separate islands in the distribution of more distinct cell states (e.g. *TREM2*⁺ macrophages[6] and *PALLD*⁺ SMCs[7]), indicated by the marker gene expression in the 3-month HFD mouse samples (Figure 3). We can also see how the endothelial-to-mesenchymal transition (EndMT) marker gene *LRG1*[8] expression within the lesion lipid core increases as the disease progresses (Figure 3 B). The difference in gene expression localization is clear when comparing *LRG1* to the common EC marker gene *CDH5*[9] in the 3-month HFD mouse sample (Figure 3 C).

References
1. Libby, *et al.*, 2019. Atherosclerosis. *Nature Reviews Disease Primers*. 2. Wolf, *et al.*, 2018. **SCANPY: large-scale single-cell gene expression data analysis.** ***Genome Biology***. 3. Pham *et al.*, 2020. stLearn: integrating spatial location, tissue morphology and gene expression to find cell types, cell-cell interactions and spatial trajectories within undissociated tissues. *biorxiv*. 4. Chistiakov, *et al.*, 2017. CD68/macrosialin: not just a histochemical marker. *Laboratory investigation*. 5. Cuff, *et al.*, 2001. The adhesion receptor CD44 promotes atherosclerosis by mediating inflammatory cell recruitment and vascular cell activation. *Journal of Clinical Investigation*. 6. Cochain, *et al.*, 2018. Single-Cell RNA-Seq Reveals the Transcriptional Landscape and Heterogeneity of Aortic Macrophages in Murine Atherosclerosis. *Circulation*. 7. Jin, 2011. The actin associated protein palladin in smooth muscle and in the development of diseases of the cardiovasculature and in cancer. *Journal of Muscle Research and Cell Motility*. 8. Song & Wang, 2015. The role of TGFβ1 and LRG1 in cardiac remodelling and heart failure. *Biophysical Reviews*. 9. Sauteur, *et al.*, 2014. Cdh5/VE-cadherin Promotes Endothelial Cell Interface Elongation via Cortical Actin Polymerization during Angiogenic Sprouting. *Cell Reports*. 10. Cuff, *et al.*, 2001. The adhesion receptor CD44 promotes atherosclerosis by mediating inflammatory cell recruitment and vascular cell activation. *Journal of Clinical Investigation*. 11. Alencar, *et al.*, 2020. Stem Cell Pluripotency Genes Klf4 and Oct4 Regulate Complex SMC Phenotypic Changes Critical in Late-Stage Atherosclerotic Lesion Pathogenesis. *Circulation*. 12. Ravindran, *et al.*, Unpublished data.

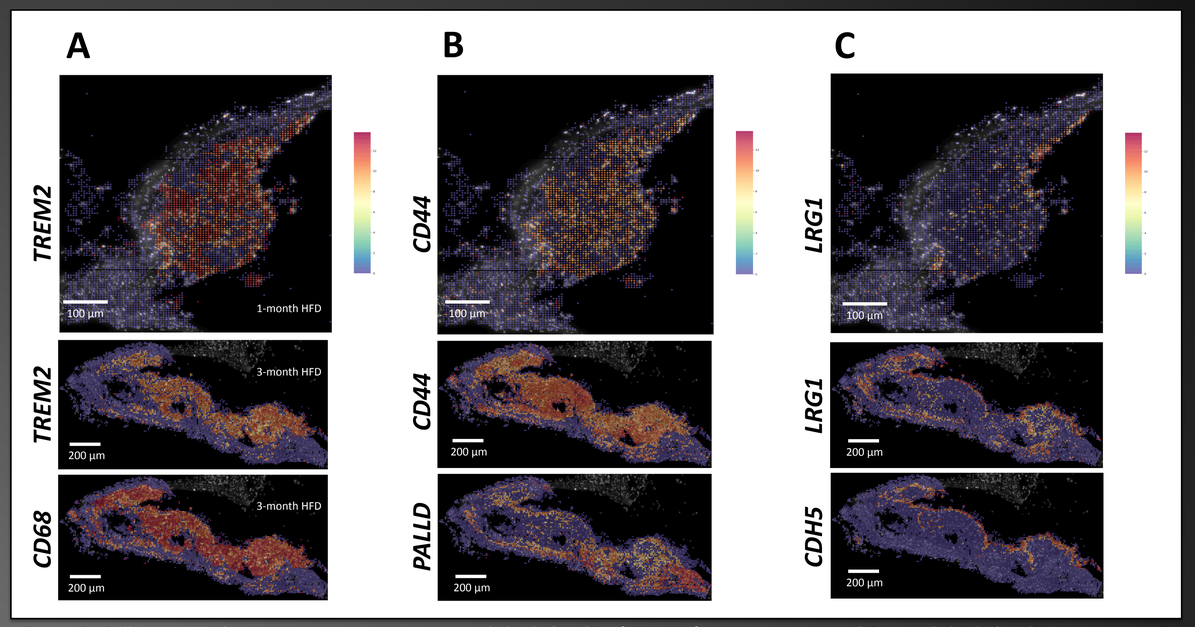

Figure 3: Cell state marker gene expression in 1-month high-fat diet (top row) mouse artery and 3-month high-fat diet mouse aortic root (middle and bottom rows). A: macrophage marker gene expression (*CD68* & *TREM2*). **B:** SMC marker gene expression (*PALLD* & *CD44*). **C:** EC marker gene expression (*LRG1* & *CDH5*) In the 1-month high-fat diet sample each dot has a diameter of approximately 6 µm, whereas in the 3-month high-fat diet sample each dot has a diameter of approximately 8 µm.

Trajectory inference analysis

Using STLearn we also analyzed the spatial pseudotime trajectory of three SMC cell states in the 3-month HFD mouse aorta cross-section (Figure 4). The three cell states are localized in three distinct areas of the diseased tissue: lipid core (blue cluster), lesion base (red cluster) and media (purple cluster). When transitioning from the medial layer to the lesion base the expression of disease-associated marker genes *CD44*[10] and *KLF4*[11] increases, while the expression of hypothesized early SMC dedifferentiation marker *ITIH4*[12] decreases (Figure 4 C). When moving further into the lipid core, we can see the levels of several disease-associated genes are elevated (Figure 4 D). In addition to genes commonly expressed in the dedifferentiated SMC states, we additionally see an increased expression of several genes marking disease-associated macrophage cell states (e.g. *TREM2* and *CD68*).

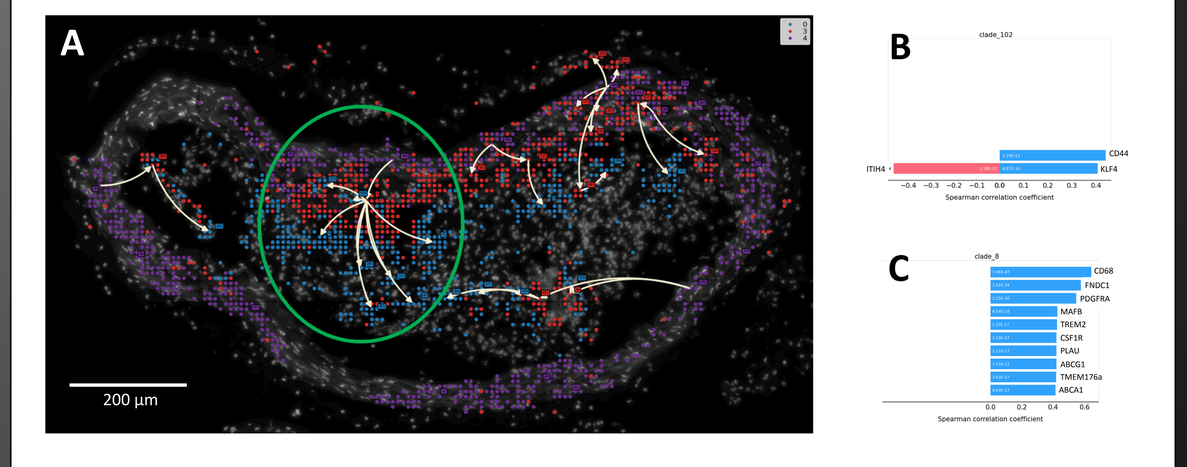

Figure 4: Trajectory inference analysis of SMC cell states in mouse aorta cross-section using STLearn. A: The pseudotime trajectories were drawn from the contractile SMC subclusters (purple cluster) to the transitioning SMC subclusters (red cluster), and finally to the dedifferentiated SMCs (blue cluster). The gene expression changes were analyzed from the trajectories within the green circle. **B:** Gene expression changes when moving from contractile SMCs into transitioning SMCs. **C:** Gene expression changes when moving from transitioning SMCs into dedifferentiated SMCs. Each dot has a diameter of approximately 8 µm.

Discussion

Using the Resolve Biosciences Molecular Cartography spatial transcripotmics platform we were able to use several bioinfromatics tools to track the distribution, development and evolution of disease-associated cell states contributing to the atherosclerosis disease progress. Our results provide a look into the gene expression fluctuations during lesion formation in a spatial context and show how different cell types and states find their place in the disease environment. We also provide a novel viewpoint for existing knowledge, which we hope will inspire new inputs into atherosclerosis research.

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