Investigating the Role of miR-26b in Vascular Calcification using Single-Cell RNA sequencing

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Background and Aims of the Project

Vascular calcification (VC) is a cell-mediated process whose central role in its pathogenesis is the acquisition of osteoblast-like phenotype by healthy vascular cells in response to pro-calcific. They are likely dependent on the cell type, the vascular bed, the VC type and the presence or absence of underlying diseases. Calcifying the internal part of the vessels (intima) is partly driven by a phenotypic transition in endothelial cells (ECs). In contrast, medial calcification is caused by a breakdown of the extracellular matrix and phenotypic change of the vascular smooth muscle cells (VSMCs). Despite recent advances, the mechanisms driving the early aspects of this vascular-to-osteoblast-like cell transition still need to be fully understood. Caporali's lab found new evidence implicating microRNa-26 (miR-26) causal role in regulating endothelial cell phenotype and contributing to vascular calcification. Following pilot data in vitro, the lab also generated miR26 global knock-out mice (miR26KO), spontaneously developing a calcific phenotype in the aorta. The main aim is to explore how the loss of miR-26b promotes the deregulation of signalling to evoke vascular calcification in the aorta and the contribution of different cell types to this process.

During my summer placement, the main aim of my project was to identify the signalling network and the cell contribution during vascular calcification in miR26KO using the data analysis platform Cellenics, an open-source analytics tool for single-cell RNA sequencing.

Methods Used

- Data Processing: Using the data obtained from the dissection of the aorta of 3 male wild-type mice and 3 male miR-26 knockout, single-cell sequencing was analysed using the software Cellenics, as observed in Figure 1. To do so, the data was first processed by filtering it by class, mitochondrial content, and the number of genes vs. unique molecular identifiers (UMIs). Moreover, it was also filtered by doublet, the data was integrated, and the embedding was configured.

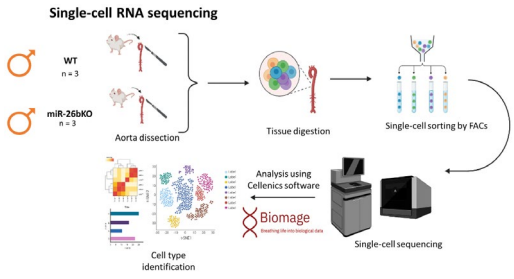

Figure 1- Summary of the Single-cell RNA sequencing process, from dissection of the tissue to cell type identification

- Cell identification by clusters: Cells were identified and organised by custom clusters using automatic Seq-type (for both mice Heart and mice Immune genes) and literature search-based marker genes.

- Identification of Subgroups of Immune Cells: Using literature research, subgroups within the 3 main groups of immune cells were identified to observe how the subgroups would behave in the knockout model. This was done through the previous identification of marker genes for each sub-group.

- Potential target identification and analysis: Previously identified targets by the lab and targets identified overlapping the genes from Cellenics and the original RNA-seq bulk were analysed using the "gene expression" section of the software Cellenics.

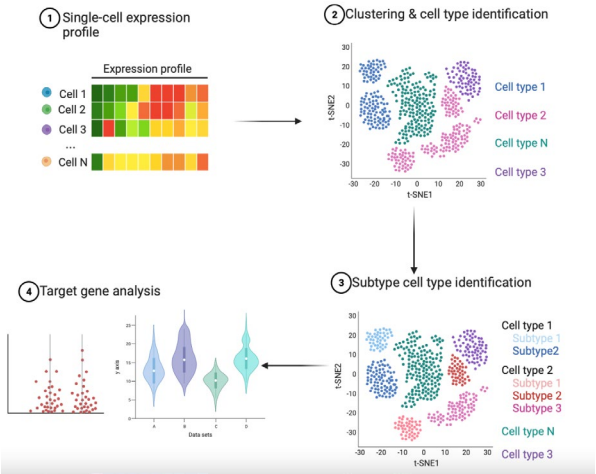

Figure 2- Summary of the Methods used for clustering and cell type identification

Results and outcomes

- Initially, 6 main clusters were identified: Smooth Muscle Cells (SMC), Endothelial Cells (EC), Fibroblasts, T cells, B cells and Macrophages, as seen in Figure 3A. When compared between wild-type and Knockout models, it was found that B and T cell populations decreased in the KO, while Macrophages and EC were increased, as can be observed in Figure 3B.

- Identification of Immune cell subgroups was performed. Using the markers genes specified in Figure 4A, 7 immune cell subgroups were identified: M1 and M2-like macrophages, Cytotoxic T cells, Th1, Th2, Th17 and Treg T cells. As observed in Figure 4B, the groups that presented the most significant change between samples were macrophages, both M1 and M2, which presented an increase of proportion in KO compared to WT. Moreover, B cells and CD8+ groups were significantly decreased in KO.

- Although an overlap between the bulk data and the data obtained from the software was done to find specific downregulated targets, no significant targets were found.

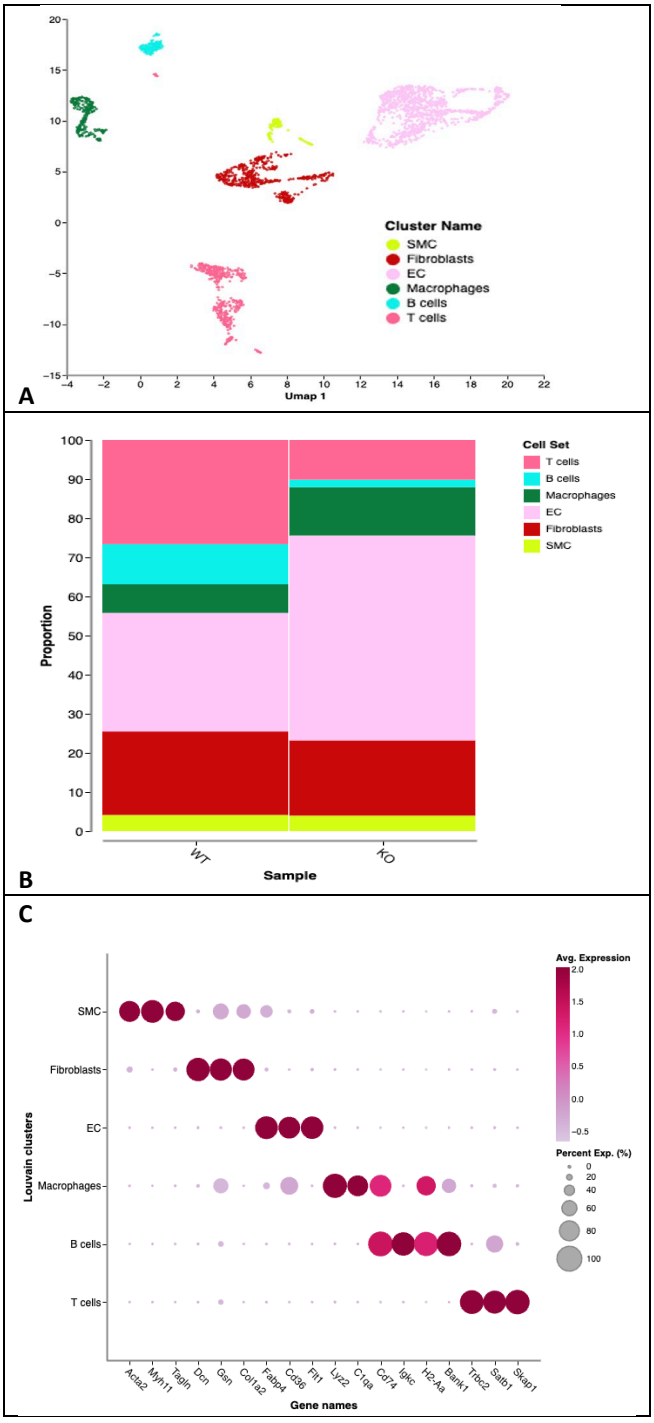

Figure 3- (A) Clustering of cells by the 6 main groups identified. (B) Proportion of the cell groups in Wildtype and miR26KO mice. (C) Marker genes expression of the cell groups.

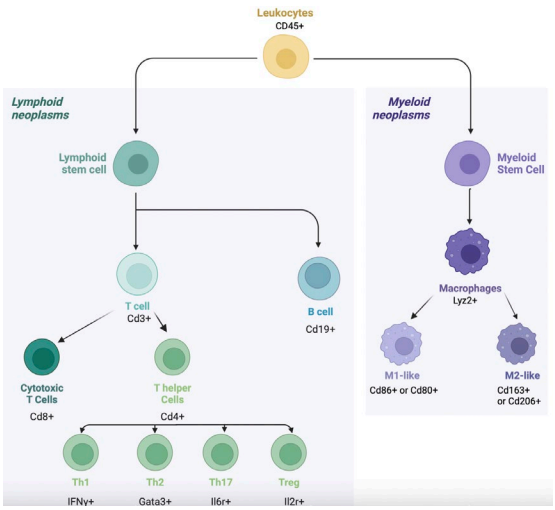

Figure 4- Final classification of the Immune Cells with the indication of the main marker genes (in black) for each type

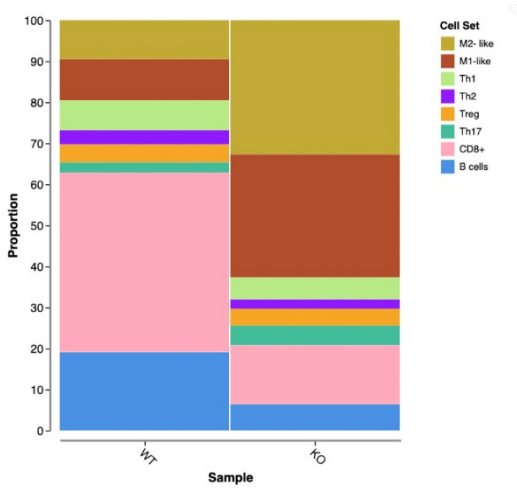

Figure 5- Proportion of the final immune cell groups in Wildtype and miR26KO mice

Future Directions in which the project could be taken

The next possible steps could be validating the identified targets by immunohistochemistry in the aorta of WT and miR26KO mice and analysing the images using ImageJ. Moreover, using R studio, a further overlapping the data obtained from single-cell sequencing and the original RNA-seq bulk of raw data could be done to identify more targets.

Departures from original project plan

To find further targets, an overlap between the genes obtained from the single-cell sequencing using the software and the original RNA-seq bulk was done. Although this was not on the original plan, the group suggested having more targets to compare to see how this would behave in the different cells' groups could be interesting.

Value of studentship to the student and to the research group

Through my work on this project, I gained a range of transferable and technical skills that will be useful for my future career in research as I plan to progress into a Master's Programme of Research, hopefully. Specifically, I acquired technical skills in bioinformatics, particularly in analysing scRNA-seq data using the Cellenics platform. This is an essential skill for any researcher working in genomics or transcriptomics. My work was incredibly valuable for the research group because, for the first time, we identified and characterized changes in the immune cell populations in this mouse model. This can open a new line of research never explored before.

Furthermore, I improved my presentation and writing skills thanks to the feedback and guidance from my supervisor and other members of the Caporali lab. Effective communication is a crucial skill for any researcher, and I believe that the opportunity to present my work and receive constructive feedback will be invaluable in improving my ability to communicate my research findings effectively.

Overall, I believe that the skills I gained through this project will be highly transferable and useful for my future career in research.

Figure 6- Photograph of the student Rosa Bauzà Sansó and the supervisor Andrea Caporali in front of the Queen Medical Research Institute in Edinburgh