Uncovering Reparative Cardiac Fibroblasts activation following myocardial infarction

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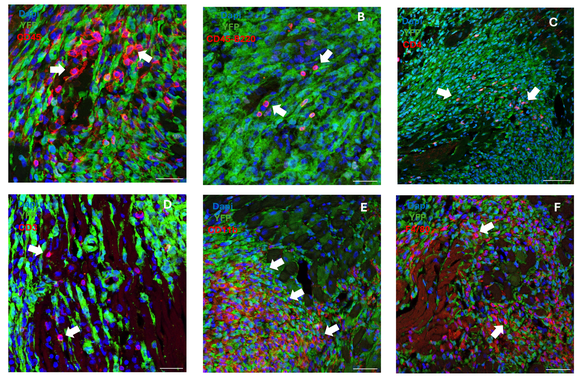
Introduction

Following **Myocardial Infarction (MI)**, activated Cardiac Fibroblasts (CF) play a pivotal role in heart tissue remodelling by initiating a fibrotic response. During this process, these cells proliferate and produce large amount of extracellular matrix (ECM) to replace necrotic tissue with a fibrotic scar. A distinct CF subpopulation, known as **Reparative Cardiac Fibroblasts (RCFs)**, emerges between days 3 and 5 post-MI. RCFs are characterized by a high expression of the ***Cthrc1*** gene, along with the specific upregulation of ECM-related genes. Although the precise mechanism of RCF activation remains unknown, their involvement in scar formation and appearance during the post-inflammatory phase of MI, suggests a potential interaction of inflammatory cells and activated CFs in the rise of RCFs.

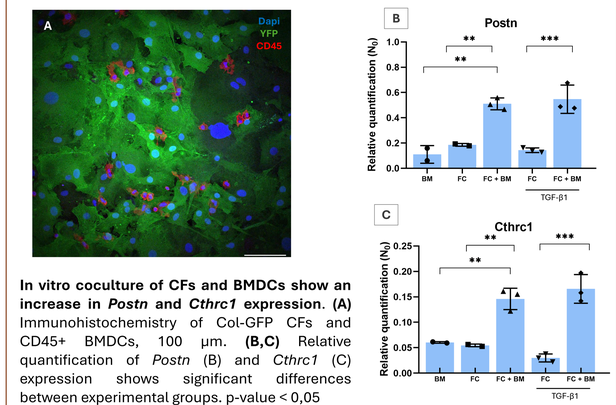
1. Spatial localization of inflammatory cells in the infarct region

Materials and methods (I): Wt1Cre-YFP mice were submitted to LDA and hearts were harvested for histological analysis of epicardial-derived CF and inflammatory cells.



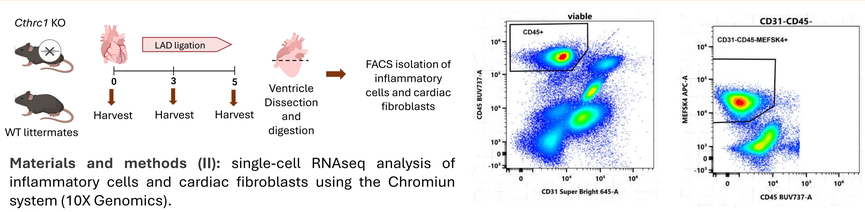
Inflammatory cells & CFs are located in the infarcted region at 5dpi. Different markers have been used to identify populations of inflammatory cells in the infarcted region of 5dpi Wt1-YFP mouse hearts. YFP+ reporter was used to identify epicardial-derived cardiac interstitial cells. (A) CD45; (B) CD45-B220; (C) CD3; (D) CD4; (E) CD11b; (F) F4/80. A,B,D: 30 µm; C, E, F: 40 µm.

2. In vitro coculture of CFs and Bone Marrow Derived Cells

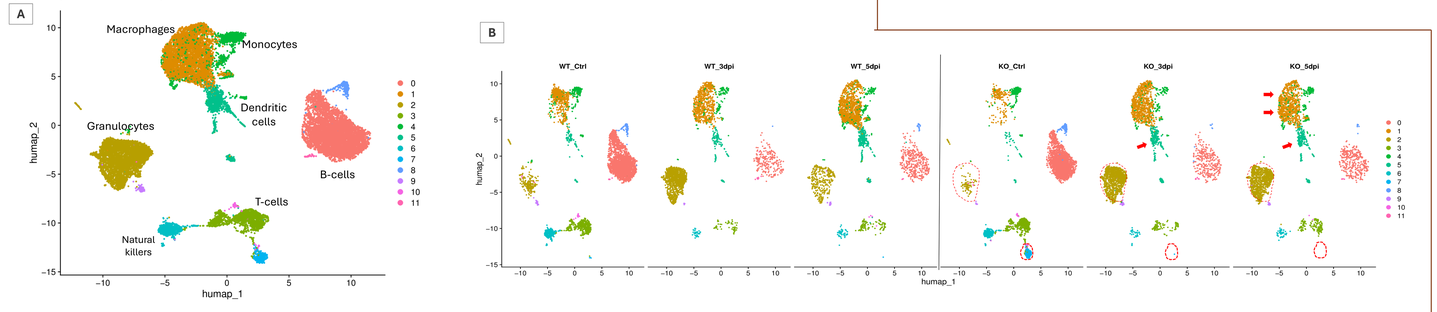


In vitro coculture of CFs and BMDCs show an increase in *Postn* and *Cthrc1* expression. (A) Immunohistochemistry of Col-GFP CFs and CD45+ BMDCs, 100 µm. **(B,C)** Relative quantification of *Postn* (B) and *Cthrc1* (C) expression shows significant differences between experimental groups. p-value < 0,05

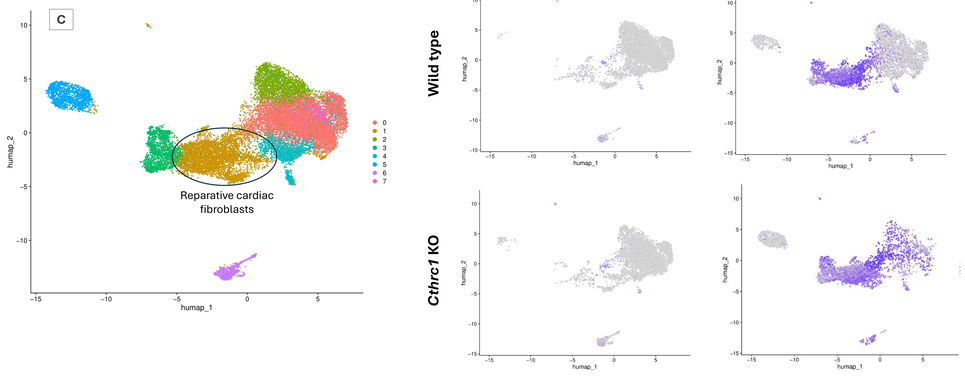
3. Single-cell analysis of inflammatory cells and cardiac fibroblasts in post-MI tissues



Materials and methods (II): single-cell RNAseq analysis of inflammatory cells and cardiac fibroblasts using the Chromiun system (10X Genomics).

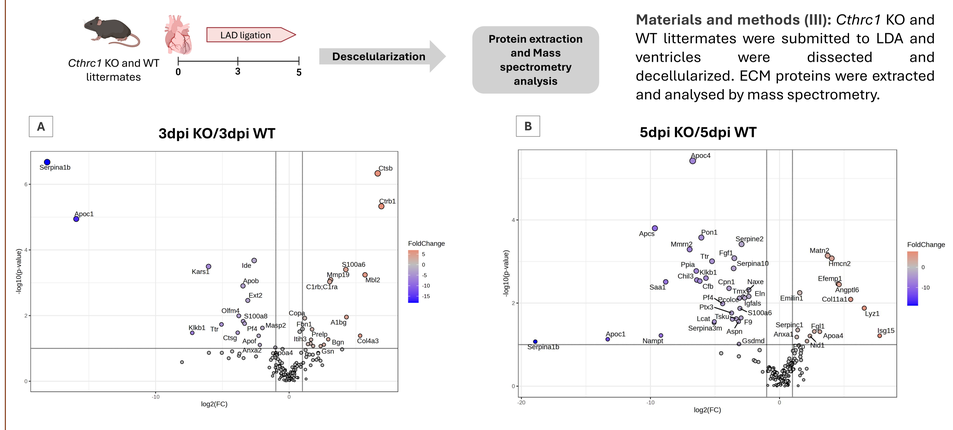


Cthrc1 depletion affects inflammatory cells dynamics after myocardial infarction. (A) UMAP plot showing inflammatory cells clusters of the integrated single cell transcriptomic profile of all the conditions used (healthy heart, 3dpi and 5dpi hearts from Cthrc1 KO or wild type mice). A total of 12 clusters were obtained and classified as: granulocytes (9,2), macrophages (1), monocytes (4), dendritic cells (5), natural killers (6), T-cells (3, 7, 10) B-cells (0, 8, 11). **(B)** Representation of individual UMAPs from each condition analysed. Arrows and dashed lines display changes in clusters 1, 2, 5 and 7 of KO mice in comparison to WT conditions.



***Cthrc1* deficiency alters *Postn* expression in homeostatic Cardiac Fibroblasts. (C)** UMAP plot showing cardiac fibroblasts clusters of the integrated single cell transcriptomic profile of all the conditions used (healthy heart, 3dpi and 5dpi hearts from Cthrc1 KO or wild type mice). **(D)** *Postn* expression across all clusters in healthy and 5dpi conditions from wild type and *Cthrc1* KO mice. **(E,F)** Immunohistochemistry from 5dpi Collagen1α1-GFP hearts from Wild type (E) and Cthrc1 KO (F) mice. E, F: 100 µm.

4. ECM remodelling after myocardial infarction



Materials and methods (III): *Cthrc1* KO and WT littermates were submitted to LDA and ventricles were dissected and decellularized. ECM proteins were extracted and analysed by mass spectrometry.

***Cthrc1* depletion interferes with ECM remodelling after MI. (A, B)** Volcano plot representation of dysregulated proteins of ECM from 3dpi (B) and 5dpi (C) Cthrc1 KO and wild type animals.

Conclusions

- After MI, inflammatory cells are found to distribute over the infarcted region, along with epicardial-derived CF.
- *In vitro* cocultures reveal that BMDCs upregulate markers of RCFs among CF.
- scRNA-Seq analysis of inflammatory cells after MI in Cthrc1-null animals reveals significant gene expression changes in several cell clusters. Postn expression in CFs from Cthrc1-null mice extends to homeostatic cardiac fibroblasts.
- Results from Cthrc1-null mice suggest a downregulation of proteins associated with ECM organization and an upregulation of proteins associated with hemostasis.

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