Single cell RNA-seq analysis reveals involvement of the interferon-I pathway and cellular senescence in calcific progression of human aortic valve cells

Doctor Ferrari SF1, Unknown Agrifoglio MA1, Unknown Manteiga JM2, Unknown Giannese FG2, Doctor Pesce MP1
Centro Cardiologico Monzino, Milan, Italy
San Raffaele Hospital, Centre for Omics Sciences, Milan, Italy

Funding Acknowledgements: Type of funding sources: Public grant(s) – National budget only. Main funding source(s): Ricerca corrente

Introduction: Valve interstitial cells (VICs) are heterogeneous fibroblast-like cells populating the aortic valve (AoV), involved in the extracellular matrix homeostasis. Under pathological conditions, VICs acquire the phenotype of calcific cells causing calcific aortic valve disease (CAVD) [1].

Purpose: In order to find distinctive traits of the phenotype in calcific VICs, we employed single cell RNA-sequencing (scRNASeq) to compare the gene expression signatures of VICs from stenotic AoVs (sVICs) vs. valves with insufficiency (iVICs), a pathology characterized by a lower level of calcification.

Methods: Before being used for scRNAseq profiling, VICs from the two pathological settings were amplified for three passages, after which they were loaded into the Drop-seq platform that encapsulated them in oil droplets with lysis buffer and barcoded primer beads. Beads were then subjected to reverse transcription, followed by library preparation. Libraries were sequenced with a coverage ranging from 50,000 to 100,000 reads per cell.

Results: A total of 5844 iVICs and 5610 sVICs were successfully separated and barcoded. Four independent VICs donors/pathology were processed and UMAP representation/clustering of cells coming from the eight samples identified the existence of 8 distinct cellular phenotypes, including embryonic progenitor mesenchymal (EMBVICs), ‘activated’ (ACVICs), ‘osteoblastic’ (OBVICs) and ‘quiescent’ (QVICs). The analysis of the cellular composition did not reveal substantial differences in the abundance of the different phenotypes in the two pathologies. On the other hand, differential gene expression followed by functional pathways enrichment showed a significant upregulation in sVICs vs. iVICs of genes/pathways related to Interferon-I (INF-I) in all the identified cellular phenotypes with the exception of QVICs. These included e.g for ACVICs, IFITM1 (log2FC = 1.76 P=3e-101), IFI6 (log2FC = 2.38; P = 1e-69), IFITM3 (log2FC 0.9 P = 5e-58), ISG15 (log2FC = 1.7 P= 4e-53) and IFI27 (log2FC = 1.54 P = 6e-41) genes.

Conclusions: This study reveals the involvement of the interferon-I pathway signaling in CAVD, supporting an autophagic/inflammatory/senescent phenotype switching in relevant VICs types resident in the human AoV.