Single cell analysis of fate-mapped macrophages reveals heterogeneity during atherosclerosis progression and regression

Researchers at NYU School of Medicine (New York, NY, USA) used single cell RNA sequencing to characterize the cellular states of macrophages in atherosclerotic plaques. Focusing on blood-derived monocytes, they tracked macrophage differentiation in mice during both progression and regression of atherosclerosis. These cells underwent dramatic changes and showed a spectrum of macrophage activation states with greater complexity than the binary M1 and M2 paradigm. Lin JD, et al. *JCI* 2019.

Snapshot

Research Area	Immunology	**10x Product**	**Chromium Single Cell Gene Expression Solution** • Chromium Single Cell 3′ Library and Gel Bead Kit v2 • Chromium Single Cell A Chip Kit • Chromium i7 Multiplex Kit • Cell Ranger Analysis Pipelines
Organism	Mouse		
Sample Types	Macrophages from aortic arch plaques		
Research Questions	**What are the phenotypes of macrophages derived from blood monocytes in atherosclerosis?** **What is the full spectrum of macrophage activation states during plaque progression or regression?**		

Experiment Overview

Mouse model for atherosclerosis in which plaques form and then are induced to regress

- Genetic fate mapping with transgenic mice to label blood-derived monocytes TdTomato (red)
- Adenovirus for PCSK9 gain-of-function gene to induce hypercholesterolemia in mice
- Mice fed atherogenic high-fat diet for 18 weeks
- Half of mice kept on high-fat diet for 2 more weeks (progression group)
- Other half of mice switched to chow and treated with ApoB antisense to lower plasma LDL levels (regression group)

Single cell RNA sequencing of blood-derived macrophages from aortic arch plaques

- Flow cytometry to isolate fate-mapped macrophages from atherosclerosis plaques
- Transcriptome analysis of 3157 cells from the progression group and 2198 cells from the regression group using the Chromium Single Cell Gene Expression Solution from 10x Genomics
- Macrophage RNA-seq libraries sequenced on an Illumina HiSeq 4000 instrument

Why Single Cell?

This study of macrophage heterogeneity demonstrates the use of a more inclusive approach than previously possible. Earlier studies, using only a limited number of markers, established the macrophage polarization model—that macrophages are either proinflammatory "classically activated" (M1) or anti-inflammatory "alternatively activated" (M2). Single cell RNA sequencing greatly increases the resolution of detail to determine the functional characteristics of macrophages, revealing the true spectrum of macrophage activation states.

Computational Analysis

The authors used principal component analysis to visualize the single cell RNA sequencing datasets, revealing a smooth transition pattern between macrophages from the progression and regression groups. They identified 11 distinct macrophage populations, characterized by their most differentially expressed genes (see Table 1). They also used diffusion pseudotime analysis to measure transitions in gene expression between cells and reconstruct cell development pathways at a single cell level.

Results

Single cell gene expression analysis offered the resolution needed to decipher the full spectrum of macrophage activation states during plaque progression and regression. Four cell populations (clusters 1, 4, 6, 9) showed similar frequencies in both groups and gene expression signatures associated with inflammation. The researchers found greater diversity of distinct macrophage phenotypes in progressing plaques (clusters 0, 3, 5, 10) compared to macrophages in regressing plaques (clusters 2, 8). Unexpected cell types included M2-like macrophages that were more abundant in progressing plaques (cluster 3) and a group of proliferating macrophages with stem cell features (cluster 7).

High-throughput single cell RNA sequencing allowed the researchers to analyze greater numbers of cells from aortic arch plaques. Enriching for blood-derived macrophages focused that analysis and allowed the authors to discover rare cell types. Transcriptome profiling provided a complete picture of macrophage phenotypic heterogeneity and helped identify potential targets for atherosclerosis therapies.

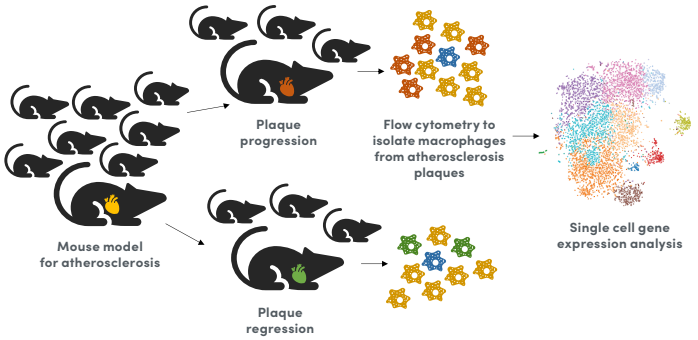

Figure 1. Study Design. Mouse model for atherosclerosis in which plaques form and then are induced to regress. Flow cytometry to isolate fate-mapped macrophages from aortic arch plaques, followed by single cell gene expression analysis.

Cell Cluster Number	Approximate Percentage of Progression Group	Approximate Percentage of Regression Group	Distinctive Gene Expression Pattern
1	23%	19%	*Folr2*
4	17%	13%	chemokines
6	16%	18%	*NMES1*
9	6%	7%	*Trem-2*
5	9%	2%	IFN signature
0	1%	<1%	*DNase113*
3	5%	2%	*Retnla* and *Ear2*
10	14%	4%	*CD74* and *MHCII*
2	0%	<1%	*Ebf1* and *Cd79a*
8	5%	31%	*HSP*
7	4%	3%	stem-like

Table 1. Single cell analysis of macrophages in plaques during atherosclerosis progression and regression. Four cell clusters (yellow) represent general inflammatory features of atherosclerosis; four cell clusters (red) were more abundant during progression; two cell clusters (green) were more abundant during regression; and one cell cluster (blue) showed stem cell-like qualities.