Single Cell Profiles of Fibroblasts Population within Murine Stellate Ganglia

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Abstract

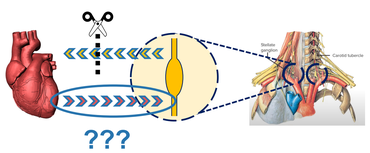
Following injuries such as cardiomyopathy, the heart tends to experience an increased in sympathetic tone. This can lead to aberrant electrical activities and elevate the patient's risk of developing future dangerous cardiac arrhythmias. While blocking the connection between stellate ganglia and the heart helps mitigate these risks, it is unknown how heart injury changes the biology of these ganglia to produce this elevation in sympathetic activity. In this project, we used single cell RNA sequencing (scRNAseq) to study the composition the stellate ganglia of six C57BL/6J (wild-type) mice, more particularly its fibroblasts. Fibroblasts were identified via the expression of *Lum* and *Smoc2*. Through unsupervised clustering, six sub-populations were obtained. Two of these clusters were excluded from further analysis because they expressed high levels of either (1) pro-inflammatory genes (e.g., *Ifit3*), or (2) markers of other cell types (i.e., *Dbi/Plp1* for glial cells). Then, after in-depth analysis of genetic markers, we identified the remaining clusters as "Immature", "Activating", "Active", and "Neurons-Interacting" fibroblasts. The "Neurons-Interacting" cluster was subsequently excluded as it needed to be further studied. Finally, we verified our findings with unbiased pseudotime analysis, which yielded a trajectory that traveled from "Immature" to "Activating" to "Active" sub-populations. Moving forward, we plan to compare these clusters with those obtained from heart-failure mice, as we aimed to elucidate a possible mechanism for how heart injury leads to an increase in sympathetic input from the stellate ganglia to the heart.

Objective

Because little is known about the presence and subtypes of fibroblasts in the stellate ganglia, we sought to investigate the diversity of fibroblasts within murine stellate ganglia using single-cell RNA sequencing (scRNAseq).

Background

- Heart receives sympathetic innervation from the **stellate ganglia** (C6 – T2). **Injured hearts tend to experience an increased in sympathetic tone.**
- Treatments aim to decrease sympathetic inputs and increase parasympathetic inputs; with the former currently being more widely used and better understood. However, not much is known regarding how signals from the heart can change the biology of the stellate ganglia.
- Stellate ganglia of patients with cardiopulmonary diseases were observed to have a slightly higher degree of fibrosis (Docimo et al., Clin Med Pathol. 2008). Although the difference was marginal, this led us to ponder the process by which the fibrosis is driven in the stellate ganglia.



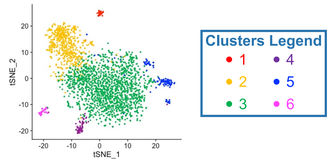
Method

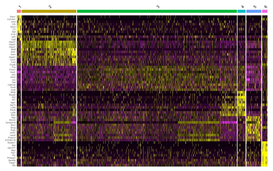
1. Isolate stellate ganglion, dissociate tissue, and sequence mRNA
2. Identify fibroblasts via expression of ***Lum*** and ***Smoc2***
3. Perform **principal component analysis**; then using 30 principal components, run unsupervised clustering with **Seurat v.4.0.2** (Hao et al., Cell 2021) and visualize the results using t-distributed stochastic neighbor embedding (tSNE)
4. Find the **identities** of each cluster by analyzing the genetic markers
5. Perform **pseudotime analysis** to see how cells move between clusters as time passes

Results

0. Unsupervised Clustering

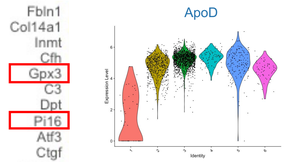
- Data from 2,596 cells (collectively collected from stellate ganglia of 6 mice), each expressing on average 5,357 ± 2,329 genes
- Obtained **6 clusters** – all observed in each mouse





1. Cluster 1 – "Immature" Fibroblasts

This cluster is characterized as the "immature" (yet to be activated) fibroblasts due to the presence of **Pi16** (a marker for undifferentiated fibroblasts), high level of **Gpx3** (higher level = younger), and low level of **ApoD** (lower level = younger).



2. Cluster 2 – "Activating" Fibroblasts

The presence of **Hsp** (heat shock proteins) and **Cxcl** and **Ccl** (chemokines) indicate that this is an injury-response population of fibroblast. Then, given that (1) the **TNFα via NF-κB** (important in macrophages action) pathway is particularly enriched, and (2) **fibroblasts are activated by macrophages**, we classify this group as "activating" fibroblasts.

Hsph1
Hspd1
Hspe1
Maff
Ifrd1
Gem
Hspb1
Cxcl1
Ccl2
Cryab

3. Cluster 3 – "Active" Fibroblasts

These cells are classified as "active" fibroblasts because they have increased expression levels for genes that are important mediators of cell cycle, differentiation, and proliferation such as **Fos, G0s2, Jun, and Junb**.

Fos
Foxs1
Vwa1
Ier2
G0s2
Jun
Oaf
Cdkn2b
Sox8
Junb

4. Cluster 4 – "Stressed" Fibroblasts

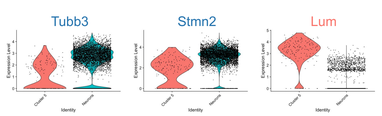
Ifit, Iigp1, and Isg15 are interferon-inducible elements (proteins, GTPases, and genes) that are upregulated with immune response. As a result, these are fibroblasts that are undergoing stress, either naturally or via the scRNAseq process (cells lysed to obtain mRNA).

Ifit3b
Rsad2
Ifit3
Ifit1
Iigp1
Isg15
Ly6a
Plac8
Ly6e
Bst2

5. Cluster 5 – "Neurons-Interacting" Fibroblasts

Many upregulated genes are believed to be specific to neurons or neuroendocrine cells: **Syt1** (synaptotagmin); **Slc6a2** (Na/NE transporter); and **Stmn2** and **Tubb3** (neuronal markers). Perhaps, these are fibroblasts that are interacting with neurons for either neuromodulation or controlling fibroblasts' growth – **need to be further elucidated**

Npy
Stmn2
Gm42418
Rtn1
Sncg
Prph
Uchl1
Lars2
Pcsk1n
AY036118

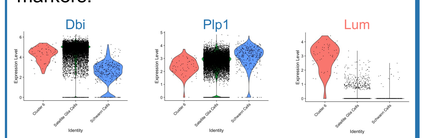


6. Cluster 6 – Glial Cells

Upregulation of glia-specific markers **Plp1**, **Fabp7**, **Dbi**, **Mpz**, and **S100b.** Levels of these markers in Cluster 6 are comparable to those of glial cells.

These ~50 cells are perhaps glial cells that are misclassified due to higher expression of fibroblast markers.

Sostdc1
Mal
Gpr37l1
Mpz
Plp1
Ptn
Pmepa1
Gpm6b
Fabp7
Dbi

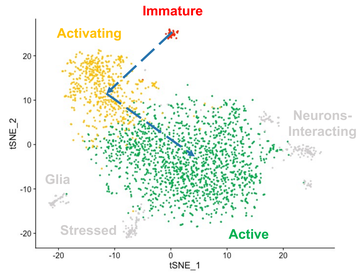


Results (continued)

7. Trajectory Analysis

Exclude Clusters 4 (Stressed Cells), 5 (Neurons-Interacting – which need to be further studied), and 6 (Glia Cells)

Using *slingshot* package, we obtain a pathway that travels **Cluster 1 (Immature) → Cluster 2 (Activating) → Cluster 3 (Active)**



Summary

- Using scRNAseq, we have identified 5 subpopulations of fibroblasts within murine stellate ganglia:
  - Cluster 1: Immature fibroblasts
  - Cluster 2: Activating fibroblasts
  - Cluster 3: Active fibroblasts
  - Cluster 4: "Stressed" fibroblasts
  - Cluster 5: "Neurons-Interacting" fibroblasts
- *We exclude clusters 6 because they are possibly glia misclassified as fibroblasts*
- Trajectory: cluster 1 (immature) → 2 (activating) → 3 (active)

Future Directions

- Identify the subpopulations of fibroblasts in mice with cardiac injury, e.g., heart failure
- Compare the subpopulations between wild-type and heart-failure mice
- **Goal: understand how heart injury changes the biology of stellate ganglion – leading to increased sympathetic tone and elevated risk of developing future dangerous cardiac arrhythmias**

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