An Integrated Mouse Spinal Cord Atlas Revealing Microglia Phenotypes in Health and Injury Conditions

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Introduction

Background:

- Microglia are <u>resident immune cells</u> in central nervous system (CNS).
- <u>Spinal cord injury (SCI) is an ideal disease model to study</u> microglia heterogeneity.
- <u>Single-cell RNA technologies</u> are powerful to study tissue heterogeneity.



Fig. 1: Microglial functions in health (left) and disease (right) conditions.

Challenges:

- Fragmented studies cause inconsistent microglia subsets annotation.
- It is challenge to retrieve scRNA-seq datasets in SCI.

Highlight results:

- The spinal cord atlas (SCA), including nearly 50,000 healthy microglia from 10 studies, enables us to study phenotyperelated microglia subsets in health and injury spinal cord.
- A scRNA-seq database was constructed to facilitate data retrieval and reanalysis of SCI research.

Future study:

- Microglia subsets will be manually subclustered and annotated.
- will be Phenotype-associated microglia subsets identified.
- Injury datasets will be mapped on the healthy atlas to identify injury-specific subsets.
- All datasets will be updated on the scRNA-seq database.

Healthy data collection and processing

Data collection:

- Metadata collected from 10 healthy studies reflects diverse phenotypes. Raw count matrix and cell types (8 of 10) were collected from data generators.
- Data processing
- Filter low quality cells & genes.
- Filter cell types that are not of interest.
- Rename inconsistent major cell type to 13 major cell types.

Diverse phenotypes for 10 healthy datasets







Fig. 2: a. Pie plots and the bar plots reflect diverse phenotype compositions including sex, spinal cord removal location, strain, and age. **b.** The bar plot showcases cell type proportions among 10 healthy datasets. Cell types from data generators were renamed and consistent to 13 major cell types.

A healthy spinal cord atlas

- The healthy atlas includes 95,745 cells and 13 cell types, 46,150 microglia (48.2%) and 1,368 myeloid cells (1.4%).
- Most microglia and myeloid are accurately labeled and can be distinguished in the healthy spinal cord atlas.







Fig. 3: a. The UMAP labeled by 13 cell types reflects cells' distribution from healthy studies. Microglia with pink dots are circled by the red box. **b.** The UMAPs reflect cells' distribution labeled by the expression of microglia markers. c. The UMAPs illustrate the distribution of cells labeled based on the expression of monocyte (Ccr2) and macrophage markers (Ms4a7). Expressions were scaled to 1-5.



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The UMAP reflects variations in microglia across datasets

 Microglia exhibit greater study-specific heterogeneity compared to myeloid cells.

Study-labeled UMAP



Fig. 4: The UMAP displays the distribution of microglia and myeloid cells labeled by 10 studies. Microglia from different data sets showing heterogenous on UMAP are circled by red box.

A scRNA-seq database for spinal cord injury

- General information for each dataset.
- Cell clustering and UMAP visualization.
- Differentially expressed gene analysis.
- GO enrichment analysis.

An example page for data general information

Dataset information

Number of identified cell types: 14

Dataset source:

Title: Microglia coordinate cellular interactions during spinal cord repair in mice

Methodology: Single cell RNAseq

Protocol: 10X Genomics

GEO/synapse number: GSE196928

Pubmed ID: 35835751

Fig. 5: The general information page e for the dataset GSM5904811 from Brennan, F., et al. (2022).



Link: https://bmblx.bmi.osumc.edu/scidl