Mafb-restricted local monocyte proliferation precedes lung interstitial macrophage differentiation

2-scRNAseq mapping and counts

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Abstract

Resident tissue macrophages (RTM) are differentiated immune cells populating distinct niches and exhibiting important tissue-supportive functions. RTM maintenance is thought to rely on either monocyte engraftment and differentiation, or RTM self-renewal. Here, we developed an inducible mouse model of lung interstitial macrophage (IM) niche depletion and repopulation to investigate IM development in vivo. Using time-course single-cell RNA-sequencing analyses, bone marrow chimeras and gene targeting, we found that engrafted Ly6C+ classical monocytes could self-renew locally in a CSF1R-dependent manner before their differentiation into RTM. We further showed that the switch from monocyte proliferation towards IM subset specification was controlled by MafB, while c-Maf specifically regulated the identity of the CD206+ IM subset. Our data shed new light on the transcriptional regulation of IM development and provide evidence that, in the mononuclear phagocyte system, self-renewal is not merely restricted to myeloid progenitor cells and mature macrophages, but is also a tightly regulated capability of mature monocytes developing into RTM in vivo.

Contents

1 Description

The Cell Ranger (v3.0.2) application (10x Genomics) was then used to demultiplex the BCL files into FASTQ files (cellranger mkfastq), to perform alignment (to Cell Ranger mouse genome references GRCm38/Ensembl97), barcode filtering, UMI counting and to produce gene—barcode matrices (cellranger count).

2 CellRanger count from fastq

The script below was used to do the mapping and counting with Cellranger.

```bash
#!/usr/bin/env bash                                          1
                                                             2
    cellranger count    --id=${id} \                        3
                --fastqs="${FastqDir}/${id}" \              4
                --transcriptome=$REF                         5
```

Here, `$id`, `$FastqDir` and `$REF` are the sample ID, the directory containing fastq files and transcriptome reference directory (mouse genome references GRCm38/Ensembl97).

3 Session information

```
sessionInfo()                                                1
```

```
## R version 4.0.3 (2020-10-10)                                               1
## Platform: x86_64-pc-linux-gnu (64-bit)                                     2
## Running under: Ubuntu 20.04.3 LTS                                          3
##                                                                            4
## Matrix products: default                                                   5
## BLAS:   /usr/lib/x86_64-linux-gnu/openblas-pthread/libblas.so.3            6
## LAPACK: /usr/lib/x86_64-linux-gnu/openblas-pthread/liblapack.so.3          7
##                                                                            8
## locale:                                                                    9
##  [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C                               10
##  [3] LC_TIME=en_GB.UTF-8        LC_COLLATE=en_US.UTF-8                     11
##  [5] LC_MONETARY=en_GB.UTF-8    LC_MESSAGES=en_US.UTF-8                    12
##  [7] LC_PAPER=en_GB.UTF-8       LC_NAME=C                                  13
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C                             14
## [11] LC_MEASUREMENT=en_GB.UTF-8 LC_IDENTIFICATION=C                        15
##                                                                           16
## attached base packages:                                                   17
## [1] stats     graphics  grDevices utils     datasets  methods   base      18
##                                                                           19
## loaded via a namespace (and not attached):                                20
##  [1] fansi_1.0.2     utf8_1.2.2      crayon_1.5.0    digest_0.6.29        21
##  [5] lifecycle_1.0.1 magrittr_2.0.2  evaluate_0.15   pillar_1.7.0         22
##  [9] stringi_1.7.6   rlang_1.0.1     cli_3.2.0       rstudioapi_0.13      23
## [13] vctrs_0.3.8     ellipsis_0.3.2  rmarkdown_2.11  tools_4.0.3          24
## [17] stringr_1.4.0   glue_1.6.1      xfun_0.29       yaml_2.3.5           25
## [21] fastmap_1.1.0   compiler_4.0.3  pkgconfig_2.0.3 htmltools_0.5.2      26
## [25] knitr_1.37      tibble_3.1.6                                         27
```