Monocytes can Proliferate in Vacant Tissue Niches prior to Differentiation into Macrophages

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 depend either on monocyte engraftment and differentiation, or on the self-renewal of mature RTM. Here, we discovered that monocytes can re-enter cell cycle and proliferate locally before their differentiation into RTM. We developed a mouse model of inducible lung interstitial macrophage (IM) depletion in which the vacant niche is repopulated by BM-derived monocytes giving rise to fully differentiated IM subsets. By performing time-course single-cell RNA-sequencing analyses of myeloid cells during niche refilling, we found that few Ly6C+ classical monocytes could self-renew locally in a CSF1R-dependent manner. We further showed that the transcription factor MafB restricted such proliferation and was essential to mediate RTM specification and identity in our model. Our data 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.

```
#!/usr/bin/env bash

    cellranger count    --id=${id} \
                --fastqs="${FastqDir}/${id}" \
                --transcriptome=$REF
```

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()
```

```
## R version 4.0.3 (2020-10-10)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 20.04.3 LTS
##
## Matrix products: default
## BLAS:   /usr/lib/x86_64-linux-gnu/openblas-pthread/libblas.so.3
## LAPACK: /usr/lib/x86_64-linux-gnu/openblas-pthread/liblapack.so.3
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
##  [3] LC_TIME=en_GB.UTF-8        LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=en_GB.UTF-8    LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_GB.UTF-8       LC_NAME=C
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_GB.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## loaded via a namespace (and not attached):
##  [1] fansi_0.5.0     crayon_1.4.2    digest_0.6.29   utf8_1.2.2
##  [5] lifecycle_1.0.1 magrittr_2.0.1  evaluate_0.14   pillar_1.6.4
##  [9] stringi_1.7.6   rlang_0.4.12    vctrs_0.3.8     ellipsis_0.3.2
## [13] rmarkdown_2.11  tools_4.0.3     stringr_1.4.0   xfun_0.28
## [17] yaml_2.2.1      fastmap_1.1.0   compiler_4.0.3  pkgconfig_2.0.3
## [21] htmltools_0.5.2 knitr_1.36      tibble_3.1.6
```