Single-cell RNA-seq shows a cell cycle alteration with a priming delay of HSCs upon aging

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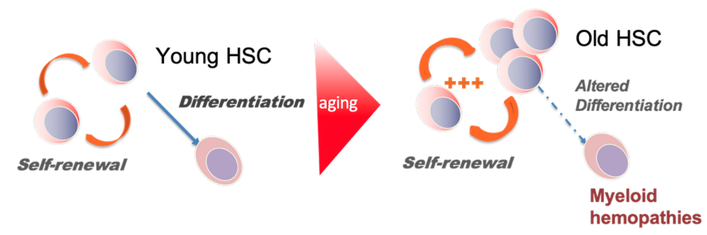
The hematopoietic stem cell aging

Introduction

Hematopoietic stem cells (HSCs) represent a rare population of cells residing in the Bone Marrow (BM) at the top of hematopoietic hierarchy. A critical balance is maintained between self-renewal and lineage differentiation of HSCs to maintain hematopoietic homeostasis. With aging, this balance is altered with an increase of `long term HSC self-renewal` and a `myeloid biased` differentiation, which favors the appearance of myeloid leukemias and anemias. My thesis project aims to understand molecular mechanisms that cause this aged-related disequilibrium.



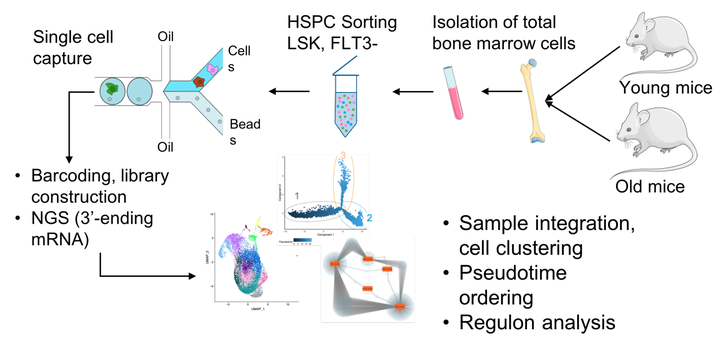
Aging of mouse HSC pool

Objectives

1. Characterize aging of early hematopoiesis in term of subpopulations and intrinsec changes of hematopoietic stem and progenitor cells (HSPCs).
2. Analyze the early differenciation process in young and old HSPCs to find its main actors (transcription factors).
3. Build a gene regulatory network to modelize HSC fate in young and old mice.

Methods

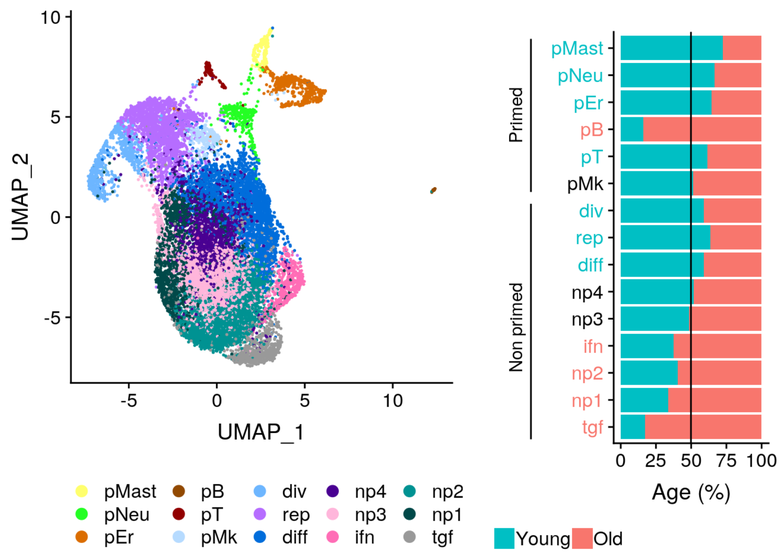
We generated single cell RNA-seq data from pools of young and old HSPCs, isolated from mouse BMs. We used Seurat (Stuart et al. 2019) and Monocle (Qiu et al. 2017) R packages to respectively cluster the cells and order them along their differentiation process. We also analyse regulon activities with pyScenic (Aibar et al. 2017) to identify transcription factor activity changes upon aging.



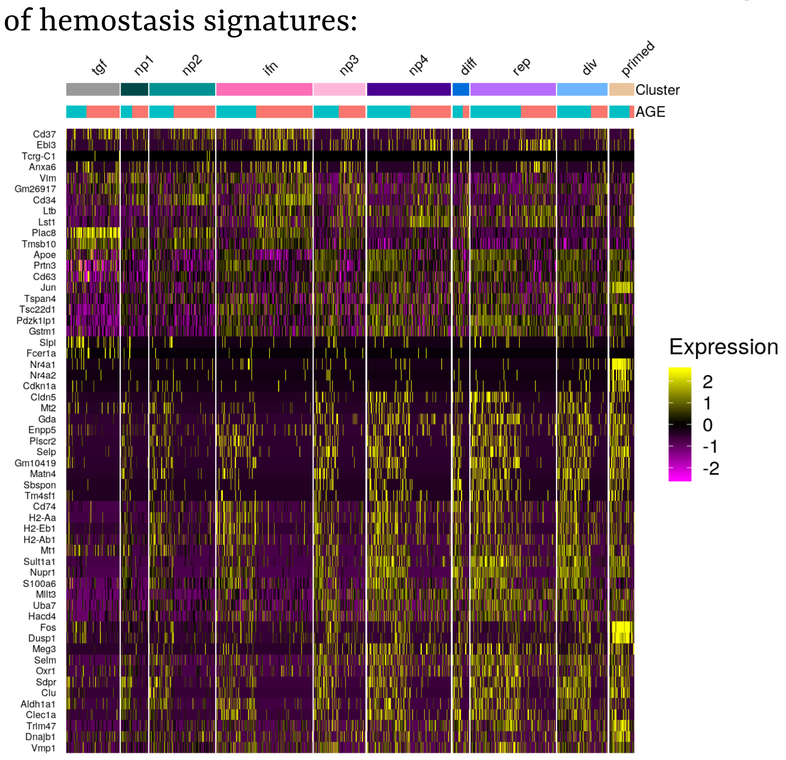
Overview of the analysis

Results

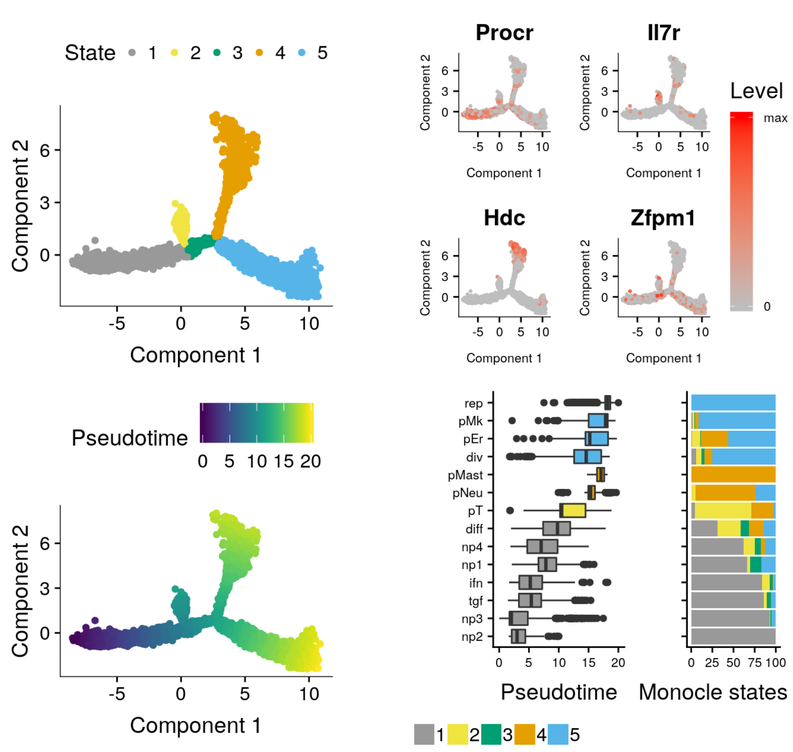
Clustering resulted in 15 clusters divided in lineage primed and non primed clusters. With aging an accumulation of HSCs to the more immature clusters is observed:



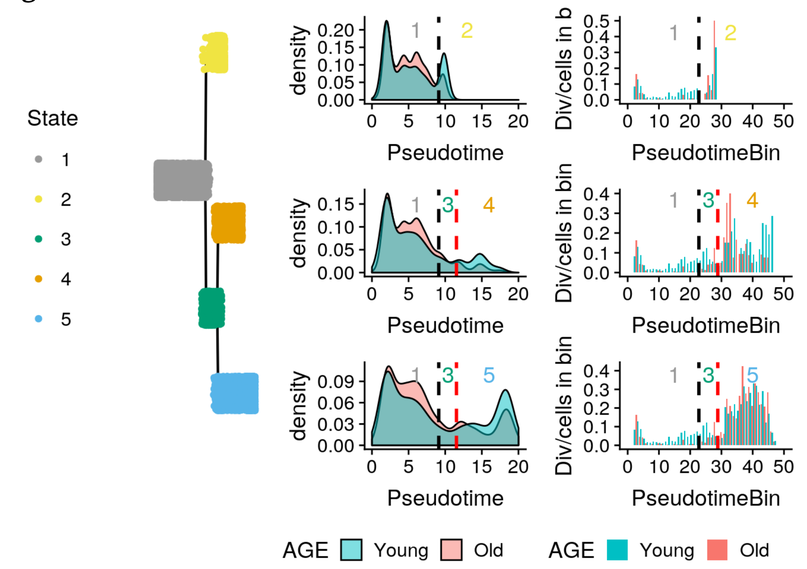
Gene expression is more altered upon aging in non-primed clusters with a lost of differentiation and a gain of hemostasis signatures:



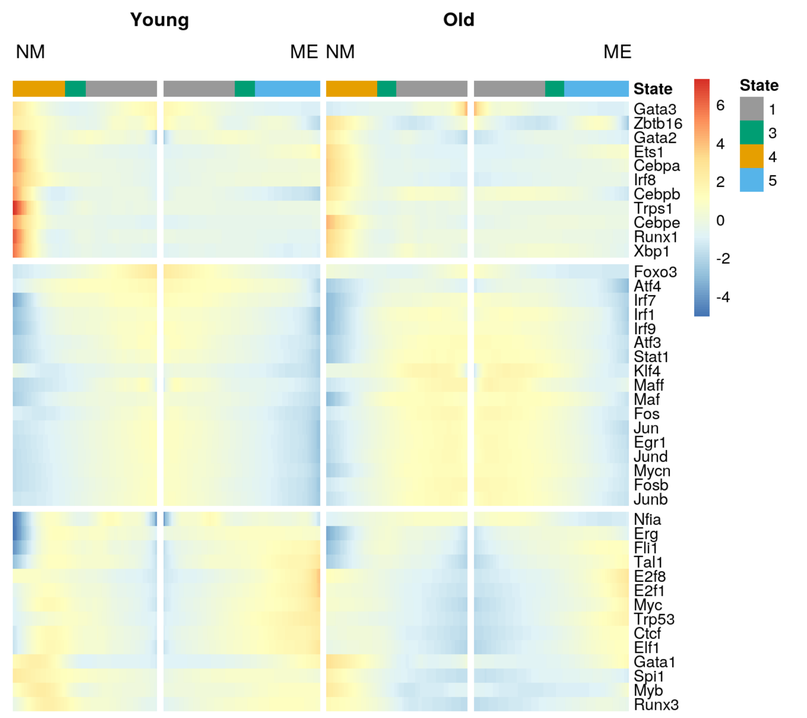
HSPC differentiation trajectory shows three paths toward primed T cells, primed neutrophils/mastocytes and primed megakaryocytes/erythrocytes:



Cell cycle analysis along pseudotime highlights a delay in differentiation associated with cell cycle slowdown in aged condition:

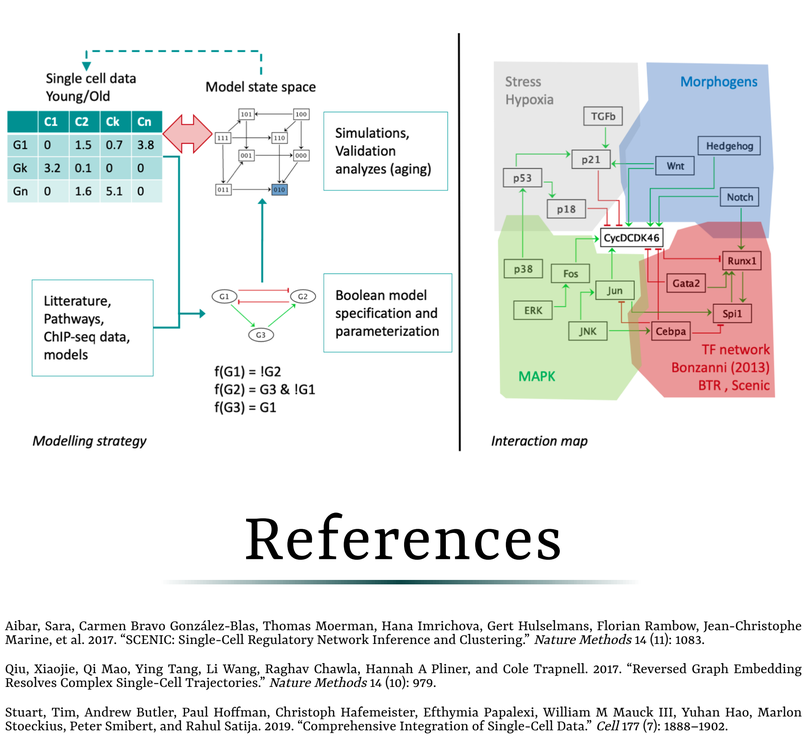


Regulon activities fit the differentiation process. However these transcription programs are altered upon aging:



Perspective

Build a boolean model of gene regulatory network explaining the molecular mechanisms that cause the aged-related alteration of HSCs in the mouse. Compare the dynamic of the obtained model with the scRNA-seq data:



References

Aibar, Sara, Carmen Bravo González-Blas, Thomas Moerman, Hana Imrichova, Gert Hulselmans, Florian Rambow, Jean-Christophe Marine, et al. 2017. "SCENIC: Single-Cell Regulatory Network Inference and Clustering." *Nature Methods* 14 (11): 1083.

Qiu, Xiaojie, Qi Mao, Ying Tang, Li Wang, Raghav Chawla, Hannah A Pliner, and Cole Trapnell. 2017. "Reversed Graph Embedding Resolves Complex Single-Cell Trajectories." *Nature Methods* 14 (10): 979.

Stuart, Tim, Andrew Butler, Paul Hoffman, Christoph Hafemeister, Efthymia Papalexi, William M Mauck III, Yuhan Hao, Marlon Stoeckius, Peter Smibert, and Rahul Satija. 2019. "Comprehensive Integration of Single-Cell Data." *Cell* 177 (7): 1888–1902.

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