Single Cell Transcriptomics

Newsletter February 2020

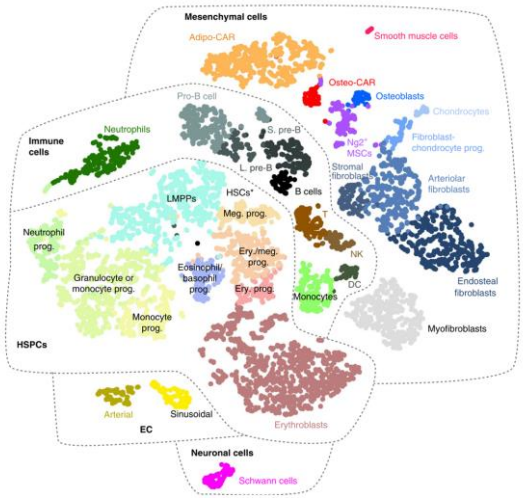
Paper 1. Baccin, C. et al. [Combined single-cell and spatial transcriptomics reveal the molecular, cellular and spatial bone marrow niche organization], *Nature 2020.*

The bone marrow is responsible for life-long blood production and skeletal regeneration, therefore scientists in this paper try to distinguish all major bone marrow (BM) populations based on single-cell RNA sequencing (scRNAseq) and spatial transcriptomics.

Using droplet-based scRNAseq 7,497 cells were analyzed and grouped into 32 clusters corresponding to distinct cell types or stages of differentiation in the bone marrow. To analyze both highly abundant and rare BM-resident cells, progressive depletion of the abundant cell types and enrichment of rare populations was performed.

For the first time origins of key cytokines responsible for BM haematopoiesis (Cxcl12 and Scf) were observed. Expression was traced towards two previously unrecognized cell subpopulations, now considered as 'professional cytokine-producing cells' and named as Osteo- and Adipo-CAR cells. Comparing sinusoidal and arteriolar niches, it was noticed that in both cases high production of stem-cell maintenance and differentiation factors takes place.

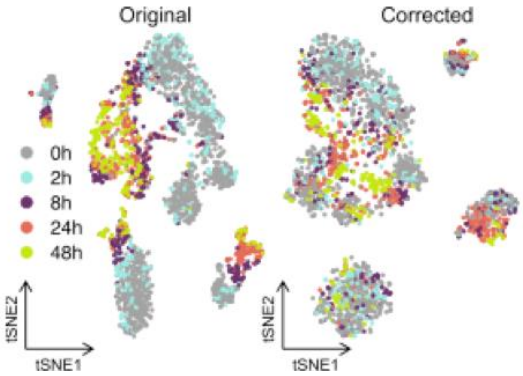
Combined spatial transcriptomics and scRNAseq reported in this paper represent one of the first systematic maps of the BM organization.



Paper 2.

Massoni-Badosa, R. et al. [Sampling artifacts in single-cell genomics cohort studies], *bioRxiv 2020.*

Blood sampling is one of the easiest ways to identify diseases and biomarkers, due to their efficiency and availability in biobanks and large clinical collections. However, the time period between sample extraction on room temperature and cryopreservation can differ between just few hours (local) and up to a few days (central). This raises the question whether such variation in conditions leads to changes in the gene expression, RNA integrity and epigenetic profiles, consequently biasing scientific findings?



The consortium led by Holger Heyn (CRG, Barcelona) investigated the effect of sampling time on scRNA-seq and ATAC (scATAC-seq) datasets. The benchmarking experiments included peripheral blood mononuclear cells from healthy individuals (PBMCs) and patients suffering from lymphocytic leukaemia (CLL). Experimental conditions included cryopreservation at different timepoints, e.g. immediately (0h) or after 2,4,6,8,24 and 48 hours.

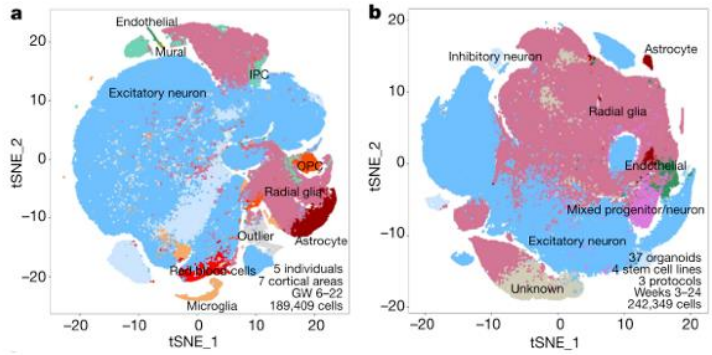
They generated 66,136 transcriptomes and 76,146 epigenome profiles for this report. As expected, gene expression varied between conditions, however gross RNA integrity and open chromatin profiles did not show any changes upon experimental conditions. Still, integrative analysis of scRNA-seq and scATAC-seq data showed fine-grained reduction for certain genes that lose open chromatin sites, leading to the fact that specific enhancer sets trigger the response to temperature changes.

Paper 3.

Bhaduri, A. et al. **Cell stress in cortical organoids impairs molecular subtype specification**, *Nature 2020.*

In the last paper, Bhaduri et al. contributed to a better understanding of human neurodevelopment and benchmarked the validity of in vitro cellular data using primary and organoid cells. Using scRNA-seq, they analyzed primary cells from seven regions in the brain resulting in 189,409 cells, comparing to data from 235,121 single cells from 37 forebrain organoids. Samples for scRNA-seq and immunohistochemistry were collected after 3,5,8,10,15 and 24 weeks of differentiation to identify cell types.

Broad cell types corresponding to radial glia, intermediate progenitor cells (IPCs), maturing neurons and interneurons were identified in both organoid and primary cell datasets. While microglia, oligodendrocyte precursors, mural and endothelial cells were identified only in brain samples. Differences also included 45% reduction in the quantity of HOPX+ cells (marker of outer radial glia (oRG)), and 63% reduction of EOMES+ intermediate progenitor cells. And the most significant reduction of 94% was noticed for SATB2+ upper-layer neurons in organoids when compared to primary samples. Investigators also proved that organoid radial glia lack specificity and that many molecular maturation programs that exist in vivo are not activated in organoids. It was also noted that organoids overexpress several cellular stress genes across all organiod cultivation protocols. This finding was confirmed by immunostaining of PGK1, ARCN1 and GORASP2. Altogether, this report raises once again a question about the validity of organoids as models of mammalian brain.



Next Single Cell Seminar

Date: 28th February 2020, Location: Mærsk Tower, Top Floor

9:00 – 9:40

Andrea Asenjo Martinez, BRIC – Identification of diseased neuronal types in schizophrenia mouse models during development

10:00 – 10:40

Erik Stensrud, 10X Genomics - Novel tools for single cell analyses: scRNA coupled with ATACseq, automatization, spatial update and more

10:40 – 11:00 Coffee and Discussion

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