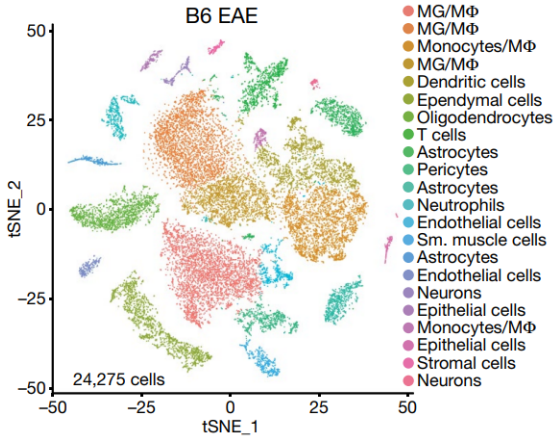
Single Cell Transcriptomics

Newsletter March 2020

Paper 1

Wheeler, MA *et al.* [MAFG-driven astrocytes promote CNS inflammation] *Nature, 2020.*

Wheeler *et al.* investigated multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE) samples using scRNA-seq (Drop-seq, Seurat/Monocle), ATAC-seq, ChIP-seq, and WGBS. Initially, they investigated 24,275 cells using scRNA-seq in WT and B6 EAE mice. In the EAE mice, they identified overrepresentation of an astrocyte subpopulation that was defined by lowered expression of *Nfe2l2* expression leading to reduced levels of the transcription factor NRF2. They proceeded to show that NRF2 suppressed pro-inflammatory astrocyte responses in the EAE model. A pseudotime analysis indicated that decreased NRF2 activation preceded increased DNA methyltransferase activity promoting inflammation. This was validated by cell culture investigations on mouse astrocytes. Ingenuity pathway analysis suggested upstream involvement of *Mafg* modulation on *Nfe2l2* activity. They confirmed involvement of MAFG by western blotting, and showed an increased number of $MAFG^{+}NRF2^{-}$ astrocytes in the EAE model. Further, ChIP-seq showed increased recruitment of MAFG to NRF2 responsive elements thereby underlining the possible pathogenic involvement of MAFG in the EAE model.

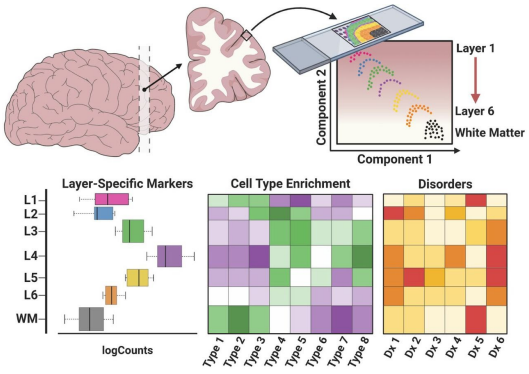


Finally, through scRNA-seq of human MS brain samples, they investigated 43,670 cells and isolated astrocytic cells. They expanded this dataset with cortical and cerebellar cells from previously published data, in total 9,673 astrocytes were considered in 48 samples (20 MS patients, 28 controls). Again, they identified an overrepresented astrocyte subpopulation in the MS samples. This subpopulation contained samples from all three patient groups, and was characterized by decreased NRF2 activation and increased MAFG activation, DNA methylation and pro-inflammatory signalling. A pseudotime analysis once again detected upregulation of *MAFG* concomitant with decreased *NFE2L2* expression thus confirming an involvement of MAFG in astrocyte-dependent CNS inflammation in MS.

Paper 2

Maynard, KR *et al.* [Transcriptome-scale spatial gene expression in the human dorsolateral prefrontal cortex], *bioRxiv, 2020*

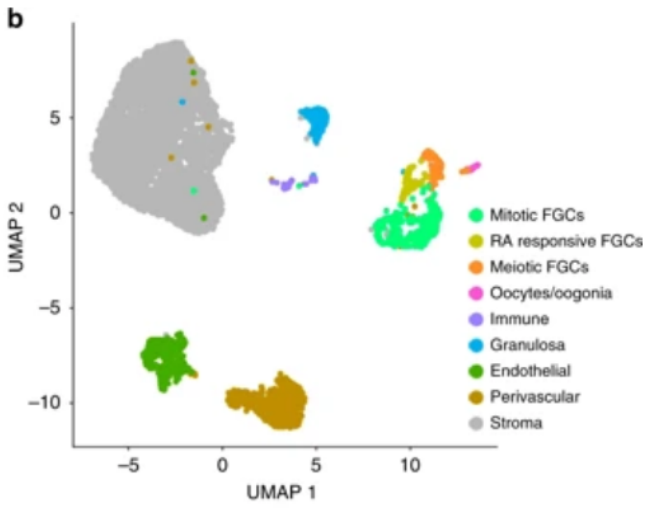
Using the 10X Genomics Visium platform, Maynard *et al.* sought to define the spatial gene expression topography of the human dorsolateral prefrontal cortex. They investigated samples from three neurotypical adult donors, in total 12 samples were investigated. Through determination of DE genes between layers, they both validated layer-specific genes from the Allen Brain Institute as well as identify genes not previously annotated as layer-specific, e.g., *AQP4* (L1), *HPCAL1* (L2), *FREM3* (L3), *TRABD2A* (L5) and *KRT17* (L6). These results were confirmed on previously published data (snRNA-seq and bulk RNA-seq) as well as novel snRNA-seq consisting of 5,231 nuclei



from two donors. Further, the authors integrated their data with disease-specific gene sets for several neuropsychiatric disorders including schizophrenia (SCZ) and autism spectrum disorder (ASD). This approach identified gene expression layer enrichment, e.g., L2 for SCZ and L5 for ASD. Of relevance to the community, they developed a data-driven framework for unsupervised cluster identification for spatial gene expression data as well as a Shiny web application to explore their data available here.

Paper 3

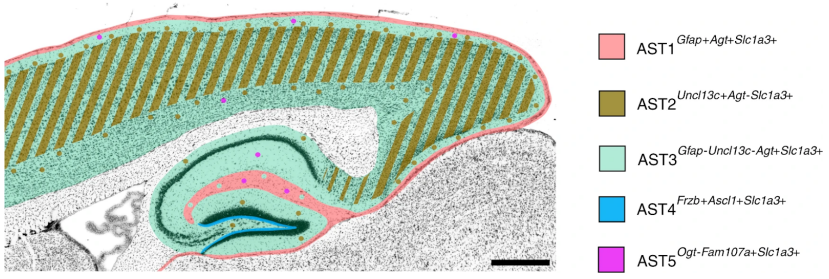
Wagner, M *et al.* **Single-cell analysis of human ovarian cortex identifies distinct cell populations but no oogonial stem cells**, *Nature Communications, 2020*



Here, the authors employed scRNA-seq and cell surface antigen profiling to investigate 24,000 cells from the ovarian cortex from 21 donors. The cells originated from cesarean sections and gender reassignment patients. They both investigated uncultured, unsorted cells and cultured DDX4$^{+/-}$ sorted cells. The main aim was to investigate the presence of oogonial stem cells, a cell type that has been postulated to exist in the ovarian cortex. Further, it was hypothesized that oogonial stem cells could be captured through DDX4 antibody isolation. After filtering, 12,160 cells remained. Wagner *et al.* identify six major cell types from the scRNA-seq data, but no oogonial stem cells. Further, the cells captured by DDX4 antibodies were identified as perivascular cells and not oogonial stem cells. This result was validated by immunofluorescence showing localization of DDX4 to the perivascular space.

Paper 4

Batiuk, MY, Martirosyan A, *et al.* **Identification of region-specific astrocyte subtypes at single cell resolution**, *Nature Communications, 2020*



Astrocytes are implied in a range of distinct processes in the brain (synapse formation, synaptic transmission, blood–brain barrier formation, metabolic support of brain cells). But whether astrocytes specialize into distinct subtypes was under debate for a long time with previous reports pointing towards limited heterogeneity of astrocytes. To shed light on this question, the authors first characterized molecular heterogeneity of astrocytes using Smart-seq2 scRNA-seq. The sensitivity of the approach allowed identification of five molecularly distinct astrocyte subtypes in adult mouse cortex and hippocampus. This was complemented by quantitative *in situ* hybridization revealing distinct layer/area-specific locations of astrocyte subtypes in the brain. Gene expression signatures also hinted possible differential functions of the subtypes. This notion was investigated using *ex vivo* calcium imaging of astrocytes that revealed their differential physiological properties. Further, combining trancriptomic and physiological data with previous data on astrocyte morphology revealed a high degree of overlap between different modalities confirming the nature of astrocyte subtypes. Differences between modalities were also present, pointing towards overlapping but distinct axes of astrocyte heterogeneity. These findings provide evidence for specialized astrocyte subtypes in the brain. The data are available here: https://holt-sc.glialab.org/.

New papers from Danish researchers

- SHARP: hyperfast and accurate processing of single-cell RNA-seq data via ensemble random projection, Shibiao Wan, Junil Kim and Kyoung Jae Won, *Genome Research, 2020*
- Gene network reconstruction using single cell transcriptomic data reveals key factors for embryonic stem cell differentiation, Junil Kim, Simon Toftholm Jakobsen, Kedar Nath Natarajan, Kyoung Jae Won, *bioRxiv, 2019*
- Mapping heritability of obesity by brain cell types, Pascal N Timshel, Jonathan Thompson, Tune H Pers, *bioRxiv, 2020*

Conferences

10x Genomics EMEA Scientific Symposium, 2th-3rd June 2020, Copenhagen, Denmark
Unraveling Cell Function Using Single-Cell Technologies, 19th March 2020, webinar by CellPress

Next Single Cell Seminar

Date: 27th March 2020, Location: Mærsk Tower, Top Floor

9:00 – 9:40
Tobias Bergmann, Institute of Clinical Veterinary and Animal Sciences, KU
The developing Entorhinal Cortex - a cellular map

10:00 – 10:40
Ulrich Pfisterer, Center for Translational Genomics, Lund University
Clinical Genomics Lund: Single Cell Facility for Translational Research and Diagnostics

10:40 – 11:00 Coffee and discussion

If you would like to announce anything single cell related, being it job announcement, event, your published paper, technology development etc, please contact us.

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