Single Cell Transcriptomics

Newsletter November 2021

Paper 1

Wälchli, T *et al*. Molecular atlas of the human brain vasculature at the single-cell level *bioRxiv, 2021 Oct.*

This preprint is a huge effort of several labs creating the first molecular atlas of the human brain vasculature using scRNA-seq. This large-scale single-cell atlas is composed of the transcriptome of 599,215 freshly isolated endothelial, perivascular or other tissue-derived cells from fetal, adult control and diseased human brain vasculature (Fig. 1). Figure 2 gives an overview of the computational analysis they performed. They found for example reactivated developmental pathways in the diseased vasculature and a loss of CNS-specific properties of the pathological endothelial cells (ECs). Instead, the pathological ECs showed an upregulation of MHC class II molecules. This human vascular brain atlas is a powerful publicly available reference for the field.

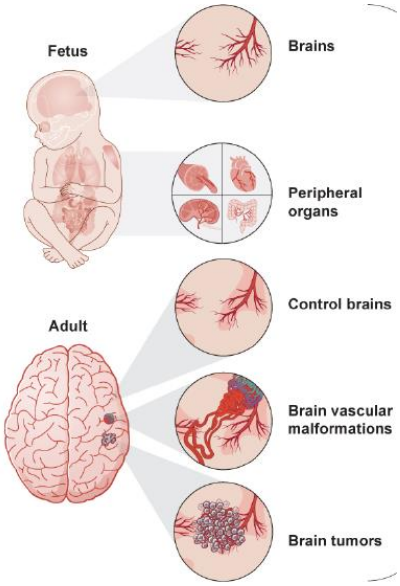

Figure 1: Fetal and adult tissue used for scRNA-seq.

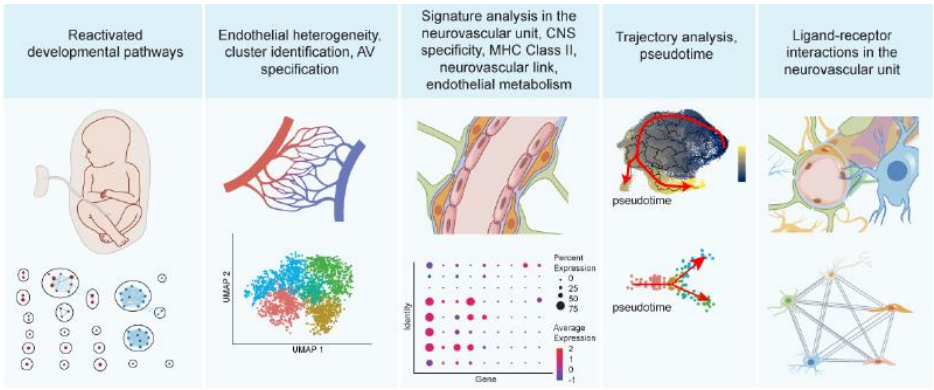

Figure 2: Summary of the computational analysis.

Paper 2

Tedesco, M *et al.* Chromatin Velocity reveals epigenetic dynamics by single-cell profiling of heterochromatin and euchromatin *Nature Biot, 2021 Oct.*

Whilst it is possible to survey open chromatin at the single-cell level with the widely used technique of scATAC-seq in this paper the authors developed a technique that probes both open and closed chromatin. Single-cell genome and epigenome by transposases sequencing (scGET-seq) uses an engineered Tn5 transposase targeting H3K9me3. The sequencing of a much larger part of the genome allows for the accurate identification of CNVs and SNVs, which was not possible with scATAC-seq. They tested scGET-seq at cancer-derived organoids and PDX models of colon carcinoma and showed that it can be used to assess the tumor genome and epigenome, illuminating paths of cancer evolution, clonality and drug resistance. Moreover, they developed Chromatin Velocity a method that identifies the trajectories of epigenetic modifications at the single-cell level.

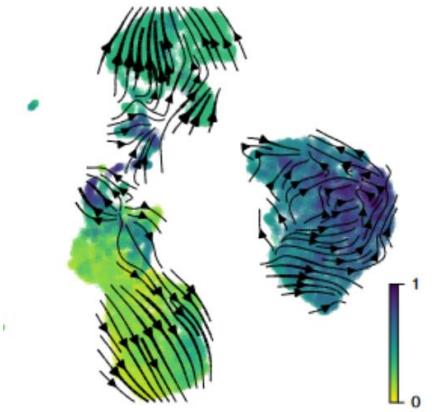

Figure 3: UMAP embedding of differentiating single cells profiled by scGET-seq. Cells are colored by velocity pseudotime, and arrow streams indicate the Chromatin Velocity extracted using scvelo.

Paper 3

Legnini, I et al. Optogenetic perturbations of RNA expression in tissue space *bioRxiv, 2021 Sep.*

The authors present here a technology that combines optogenetics and CRISPR to activate or knock-down RNA of target genes at single-cell resolution and conditional on both space and time. These perturbations experiments are necessary as a next step after for example spatial transcriptomics to dissect the molecular mechanism of cellular interaction in complex tissues. To perform light-inducible gene activations, they used split CRISPR-Cas9-based Photoactivatable Transcription System (SCPTS), which consists of an enzymatically dead Cas9 that has been split into two domains and fused to the photoinducible dimerization moieties pMag and nMag. In this system blue light activates transcription. To induce targeted RNA knock-downs they combined a SCPTS module with a CRISPR/CasRx module, so that photo-stimulation controls CasRx synthesis. To achieve spatial determined light stimulations, they tested 3 different approaches as depictured in Figure 4.

For proof of principal, they optogenetically induced SHH expression in a specific location within neural tube organoids. This robustly induced known SHH spatial domains of gene expression – cell-autonomously and across the entire organoid.

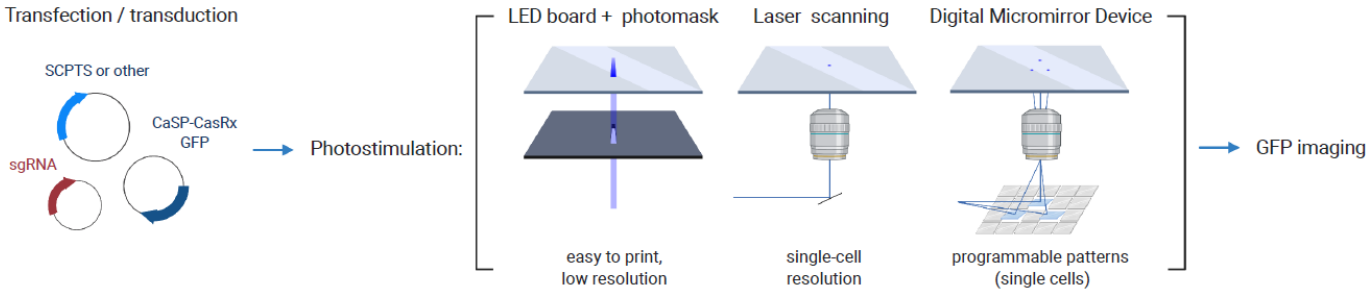

Figure 4: Options of spatially induced photo-stimulations.

Paper 4

Chung, H et al. Joint single-cell measurements of nuclear proteins and RNA in vivo *Nature, 2021 Oct.*

Authors present here intranuclear cellular indexing of transcriptomes and epitopes (inCITE-seq), a method that allows for simultaneous measurement of nuclear protein levels and the transcriptome in thousands of nuclei. This enables to relate transcription factor levels and gene expression and how this varies across context and in disease. To detect the nuclear proteins DNA-conjugated antibodies are used. Nuclei are slightly fixed with formaldehyde, permeabilized and blocked to minimize nonspecific binding of antibodies inside the nucleus, which is one of the major challenges to overcome.

The authors applied inCITE-seq on murine brain tissue after the pharmacological induction of neuronal activity. Modeling gene expression as a linear combination of quantitative protein levels revealed genome-wide associations of each TF and recovered known gene targets.

It is best to combine transcriptome data from inCITE-seq and standard snRNA-seq, because of the currently lower RNA complexity of inCITE-seq data. Nevertheless can inCITE-seq illuminate how combinations of transcription factors shape gene expression with direct applications to solid or frozen tissues.

Next Single Cell Seminar

Next seminar will take place end of November. Date and speaker are to be announced.

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

Contact: laura.wolbeck@bric.ku.dk