Single Cell Transcriptomics

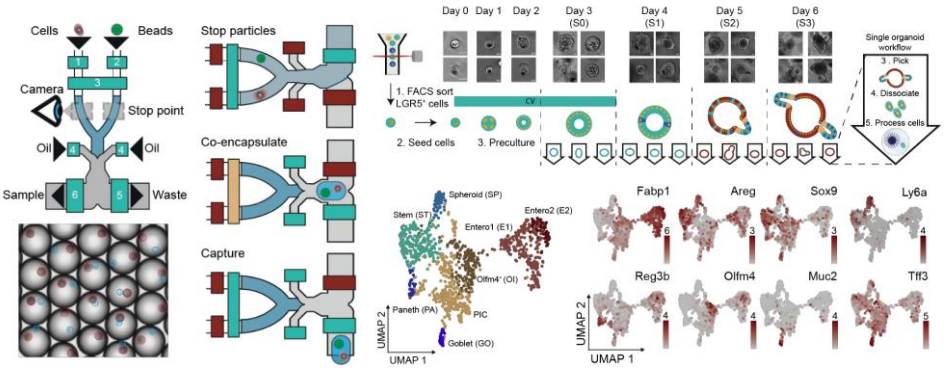
Newsletter June 2020

Paper 1

J. Bues, M. Biočanin *et al.* **Deterministic scRNA-seq of individual intestinal organoids reveals new subtypes and 2 coexisting distinct stem cell pools**, *bioRxiv, 2020.*

In this study, scientists developed a deterministic, mRNA-capture bead and cell co-encapsulation dropleting system - DisCo. Its idea is to overcome the lack of technical ability of current microfluidics-based scRNA-seq technologies limited to the samples of more than 1 000 cells. Majority of current methods are based on stochastic cell capture, entailing large sample inputs, whilst efficient processing of small tissues remains demanding. DisCo has implemented an active particle coordination which allows precise capture and co-encapsulation of one cell and one mRNA-capture bead. The advantage of the method is efficiently processing samples of 100 cells and below, the notable advancement for the single-cell research of e.g. zebrafish embryos, organisms like *C. elegans* or intestinal organoids. They analyzed 31 single organoids at 4 developmental time points.

By UMAP they visualized 1132 sample cells and proved consistency with previously published pooled organoid scRNA-seq reads-out. Moreover, no batch-based clustering was noticed after correction of batch effects, neither they detected any clustering driven by cell quality. These results support the high cell type-resolving power of DisCo.



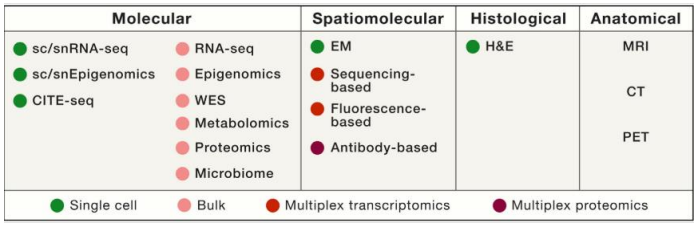
Notably, among the identified subtypes, they detected a rare phenotype predominantly comprised of precursor- and mature goblet cells – "gobloids". Additionally, another identified subtype is "spheroids", comprised of regenerative fetal-like stem cells marked by Stem Cell Antigen-1 (Sca-1/Ly6a).

Paper 2

O. Rozenblatt-Rosen, A. Regev, P. Oberdoerffer, T. Nawy *et al.* **The Human Tumor Atlas Network: Charting Tumor Transitions across Space and Time at Single-Cell Resolution**, *Cell, 2020*

The US scientists will establish the first clinical, experimental, computational and organizational framework to generate informative and publicly accessible 3D atlases of cancer transitions for a diverse set of tumour types. The Human Tumor Atlas Network (HTAN) will also provide transitions of pre-malignancy to malignancy formations, from a primary tumor to metastasis, and from pre-treatment to post-therapeutic response. Many genomic studies already led to the identification of numerous genetic drivers of malignancy, but the disadvantage of the studies is that relied on bulk profiling of advanced tumors, and most often in only one single time point with limited information about the treatment and outcomes. Luckily, by the recent advances in single-cell and multiplexed spatial analysis of tissue, the scientific community gain insight into complexity at unprecedented resolution. HTAN obtained two complementary approaches to address the cellular and spatial profiling, which pairs the single-cell profiles from dissociated specimens (transcriptome, multiplexed protein, genome-wide chromatin accessibility, or methylation) with spatially resolved multiplexed assays for RNAs or proteins in tissue.

To accomplish this goal, HTAN tumor-atlas generation is expected to involve in five interdependent steps:
(1) collection of longitudinal data from diverse modalities across multiple spatial scales ranging from subcellular to cell-cluster resolution
(2) basic processing and quality control of each data modality to ensure accuracy and reproducibility;
(3) identification of cell types, states, and positions for annotating tumor composition;
(4) identification of features for describing cell-cell interactions, intercellular communication, cell neighborhoods, and mesoscale spatial motifs;
and (5) integration of experimental and clinical datasets into a comprehensive atlas

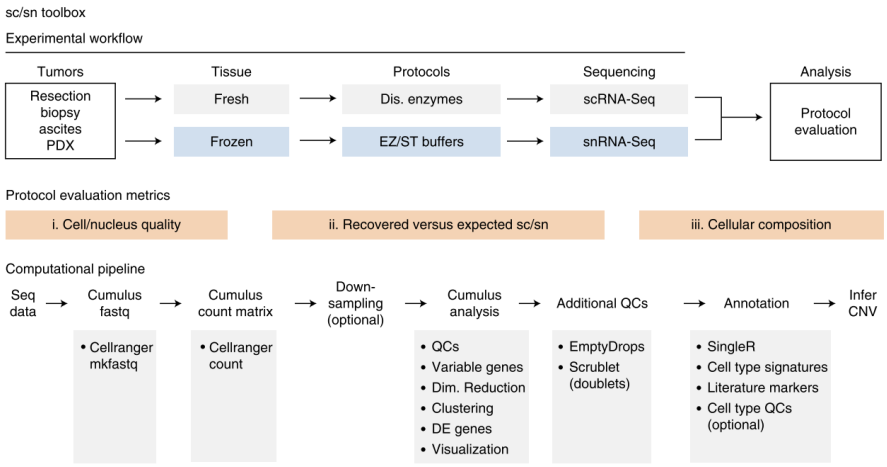


Paper 3

M. Slyper, C. Porter, O. Ashenberg *et al*. [A single-cell and single-nucleus RNA-Seq toolbox for fresh and frozen human tumors](), *Nature Medicine, 2020*

Recent advances in the single-cell RNA-Seq have addressed the complexity of cellular ecosystems of malignant and non-malignant cells. Complexity arises from the diversity and multiple interactions which affect cancer progression, drug susceptibility or resistance. Improved scRNA-seq still requires quick dissociation customized to the type of tumor, and involves enzymatic digestion, which leads to loss of cell or changes in gene expression. The clinical challenge in the method is obtaining fresh tissue which is time-sensitive and require strict coordination between the isolation of tissue and processing teams. In contrast, single-nucleus RNA-Seq process single nuclei isolated from frozen tissues, the advantage to handle samples that cannot be successfully dissociated when fresh, due to size or fragility. To address the problem, scientists developed a systematic toolbox for fresh and frozen tumor using scRNA-Seq and snRNA-Seq, in respective order.

To generalize across tumor types, they tested eight different tumor types, including the comparison of both fresh and frozen preparations the same tumor sample. In total, they analyzed 216,490 cells and nuclei across 23 tumors, from 22 patients spanning 40 sample preparations. Regarding the optimization of the protocols, they evaluated and compared protocols based on cell nucleus quality, number of recovered versus expected cells/nuclei and cellular composition. To estimate cell/nucleus quality in consideration is taken both experimental and computational metrics.

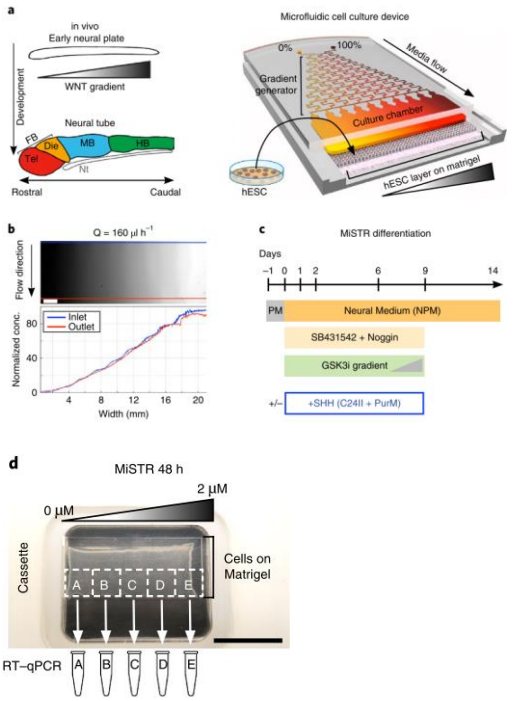


Experimentally, they measured cell viability, the extent of doublets or aggregated in the cell/nucleus suspension, cDNA quality, while computationally they evaluated the percent of reads mapping to the transcriptome, genome and intergenic regions, the number of reads, transcripts (UMI) and genes detected per cell/nucleus. The developed toolbox provides guidance for tumor studies, including criteria for evaluation and selection of appropriate methods from the toolbox for other tumors.

Paper 4

P. Rifes, M. Isaksson *et al*. [Modeling neural tube development by differentiation of human embryonic stem cells in a microfluidic WNT gradient](), *Nature Biotechnology, 2020*

Scientists from Denmark showed us the model of early human neural tube development using human embryonic stem cells by microfluidic-controlled stem cell regionalization (MiSTR). The MiSTR system is a microfluidic gradient generator establishing sequential mixture of two media containing 0 to 100% morphogen to reach a stable and linear gradient of GSK3i. Analysis of 24-48 h showed upregulation of WNT target genes (AXIN2, CCND1 and LEF1) from the lowest sample of GSK3i exposure to the highest, indicating that GSK3i gradient is translated into a WNT signalling gradient. Continuing gradient showed expression of the caudal marker GBX2 at the highest concentration of GSK3i exposure, whilst the rostral marker-OTX2 showed the opposite expression. At 48 h the expression pattern was identical to the late epiblast and presumptive neuroectoderm in the mouse model at E7.5 making grounds to use MiSTR as a reliable and predictable model to further investigate the mechanisms of human rostro-caudal regionalization.



New single cell papers from Denmark

- [Transcriptional dynamics of hepatic sinusoid-associated cells after liver injury]() Mike K. Terkelsen, Sofie M. Bendixen, Daniel Hansen, Emma A.H. Scott, Andreas F. Moeller, Ronni Nielsen, Susanne Mandrup, Anders Schlosser, Thomas L. Andersen, Grith L. Sorensen, Aleksander Krag, Kedar N. Natarajan, Sönke Detlefsen, *Hepatology, 2020*

Next Single Cell Seminar

June's seminar: to be confirmed based on update regarding KU corona-related regulations.

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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