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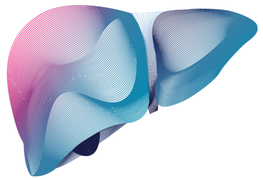

With-in patient comparison of scRNA-seq versus snRNA-seq on human transjugular liver biopsies

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Introduction

- Transjugular liver biopsies (TJB) are the only safe way to collect liver tissue in patients with ascites and/or coagulation disorders, however they have a diameter of <0.7mm [1].
- Both single-cell (scRNA-seq) and single-nucleus RNA-sequencing (snRNA-seq) are powerful tools that allow transcriptomic profiling of thousands of individual cells [2]. However, no comparison of both techniques has been made in human liver biopsies.

Aim

- To validate a protocol for scRNA-seq and snRNA-seq on human TJB's
- To compare scRNA-seq and snRNA-seq with-in patient in human liver biopsies

Method

- Patients with MAFLD/ALD liver cirrhosis (n=3) underwent a TJB, with 5 samples processed per patient (half for each technique).
- Ethical approval was obtained (S64744, University Hospitals Leuven)
- A collagenase-based protocol for scRNA-seq and a Dounce/TST-based protocol for snRNA-seq were optimized for TJB's.
- Raw sequencing reads were aligned to human reference genome (CRCh19/hg19).
- Downstream and statistical analyses were performed using R (v4.1.2, with packages CellRanger, Seurat, Doubletfinder,…) and GraphPad Prism (v9.0).
- A paired sample t-test or Wilcoxon matched-pairs signed ranked test were used to compare cell numbers and percentages

Conclusions

- A **working protocol** to use scRNA-seq and snRNA-seq was validated for TJBs.
- Both techniques **differentially detect hepatic cell types**
  - Only a small number of hepatocytes could be detected using scRNA-seq, in contrast to snRNA-seq
  - Endothelial cells and NK/T-lymphocytes were positively selected using scRNA-seq
- **Gene expression** within specific cell types **correlated highly** between both techniques.

References

1. **Sue M.J. et al.** Transjugular liver biopsy: safe even in patients with severe coagulopathies and multiple biopsies. *Clin Transl Gastroenterol* 2019; 10
2. **Ramachandran P. et al.** Single-cell technologies in hepatology: new insights into liver biology and disease pathogenesis. *Nat Rev Gastroenterol Hepatol* 2020, 8:; 457)472

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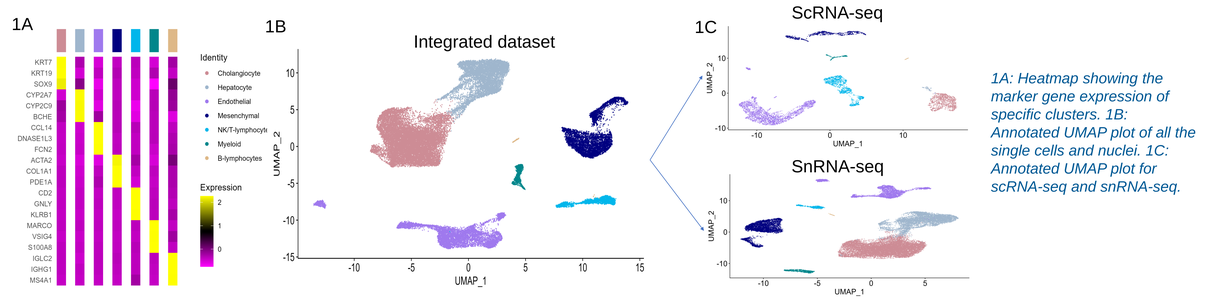
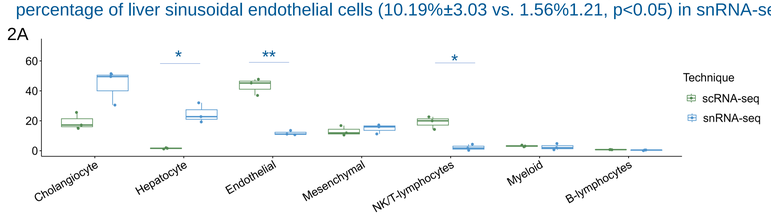
Results

- In total, 31.055 single nuclei and 6.160 single cells could be retrieved after quality control.
- 7 major cell types could be identified, that could be further subclustered into 17 different subclusters.



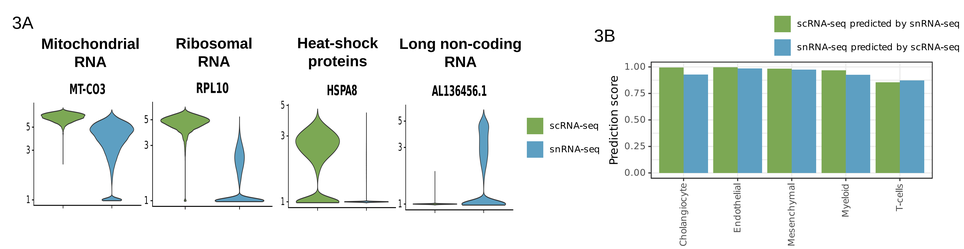
1A: Heatmap showing the marker gene expression of specific clusters. 1B: Annotated UMAP plot of all the single cells and nuclei. 1C: Annotated UMAP plot for scRNA-seq and snRNA-seq.

- There was a lower percentage of NK/T-lymphocytes (2.14%±2.04 vs. 18.93%±4.29) and endothelial cells (11.78%±1.56 vs. 43.32%±5.67), and a higher percentage of hepatocytes (24.60%±6.56 vs. 1.61%±0.4) in snRNA-seq vs scRNA-seq.
- Within the clusters, there was a lower percentage of vascular smooth muscle cells (VSMC)(20.39%±6.41 vs. 68.30%±11.30, p<0.05), hepatic artery endothelial cells (7.43%±3.75 vs. 14.32%±5.52, p<0.05) and infiltrating monocytes (8.99%±2.27 vs. 43.29%±11.25, p<0.05) and a higher percentage of liver sinusoidal endothelial cells (10.19%±3.03 vs. 1.56%1.21, p<0.05) in snRNA-seq vs. scRNAseq.



*2A: Boxplot showing the percentage of every cluster in each sample, comparing both techniques. Median and range are shown. *p<0.05, **p<0.01*

- In a direct comparison of snRNA-seq and scRNA-seq, long non-coding RNA was upregulated in snRNA-seq, and mitochondrial RNA and ribosomal RNA were upregulated in scRNA-seq. As would biologically be expected.
- Stress-related genes (e.g. heat shock proteins) were upregulated in scRNA-seq.
- Based on the gene signature of scRNA-seq, we could correctly identify the cell type of 96.9% of the nuclei and vice versa for 96.7% of the cells, with high mean prediction scores for the major cell types.
- Within subclusters, there was a high correlation of normalized RNA expression between both techniques before and after removing mitochondrial, ribosomal and long-non coding RNA expression. E.g. 0.81 and 0.93 resp. for VSMCs (Pearson correlation).



3A: Violin plot showing the expression of specific genes per technique 3B: Bar plot showing the mean prediction identity score per cluster of cells and nuclei, predicted using the gene signature of the snRNA-seq and scRNA-seq data respectively. Scores ranging from 0 to 1.