

## 1. FINAL REPORT – YEAR 2

### **Project Title: Defining the interactome of bile duct inflammation in PSC**

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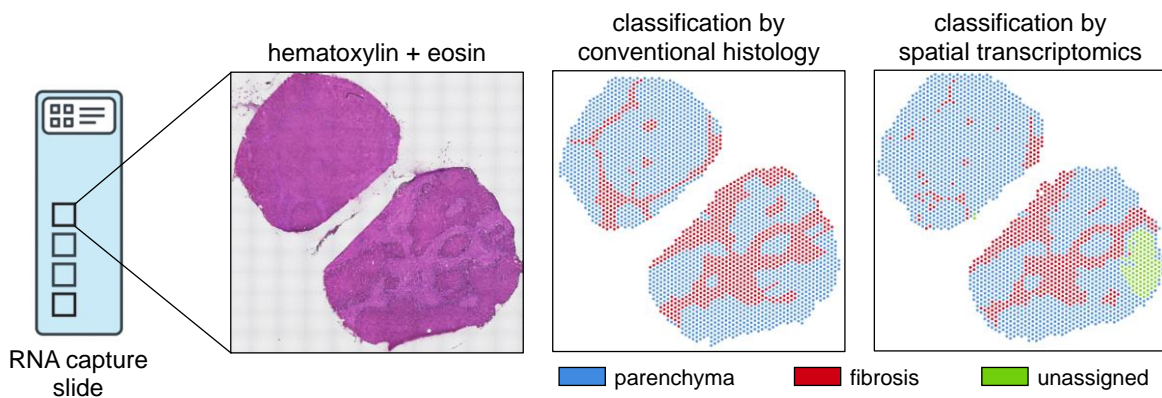
## 2. RESEACH SUMMARY

**Background:** Liver fibrosis and cirrhosis have limited therapeutic options and represent a serious unmet patient need. Recent use of single-cell RNA sequencing (scRNAseq) has identified enriched cell types infiltrating cirrhotic livers however the microanatomical localization of these lineages and their gene expression has not been clearly defined.

**Methods:** To assess if fibrotic liver regions harbour unique cell types, we analyzed end-stage explants from patients with primary sclerosing cholangitis (n=4), primary biliary cholangitis (n=2) and alcoholic liver disease (n=2) by combining whole tissue spatial transcriptomics (Figure 1), scRNAseq data and gene deconvolution analysis by CIBERSORTx. Localized enrichment of gene expression was validated at the protein level by immunofluorescence staining of the same liver sections analyzed by spatial transcriptomics.

**Results:** Spatial transcriptomics clearly identified areas of distinct gene expression in fibrotic liver regions which strongly corresponded to the total area (Spearman  $r=0.97$ ,  $P=0.0004$ ) and precise location of parenchymal and fibrotic areas as classified by conventional histology (parenchyma: 87.9% mean congruency, range: 73.1–97.1%; fibrosis: 68.5% mean congruency, range: 41.0–91.7%). Deconvolution and enumeration of parenchymal and fibrotic gene content measured by spatial transcriptomics into distinct cell states revealed significantly higher frequencies of *ACTA2+* *FABP4+* and *COL3A1+* mesenchymal cells, *IL17RA+* *S100A8+* and *FCER1G+* tissue monocytes, *VCAM1+* *SDC3+* Kupffer cells, *CCL4+* *CCL5+* *KLRB1+* and *GZMA+* *IL17RA+* T cells, and *HLA-DR+* *CD37+* *CXCR4+* and *IGHM+* *IGHG+* B cells in fibrotic liver regions compared to parenchymal areas of cirrhotic explants.

**Conclusion:** Our findings indicate that spatial transcriptomes of parenchymal and fibrotic liver regions express unique gene content within cirrhotic explants and demonstrate proof-of-concept that spatial transcriptomes combined with additional RNA sequencing methodologies can refine the localization of gene content and cell lineages in the search for novel antifibrotic targets.



**Figure 1.** Classification of parenchymal and fibrotic regions by conventional histology and spatial transcriptomics. Unassigned regions represent areas of low RNA counts as measured by spatial transcriptomics. Hematoxylin and eosin (HE) staining for assessment by conventional histological shown for reference.

### 3. ACHEIVED MILESTONES

We successful achieved all of the original milestones proposed for Aim 1, Aim 2 and generation of cholangiocyte organoids for Aim 3. ‘Immune cell-cholangiocyte organoid experiments’ in Aim 3 are ongoing and the completion of this experiments will form the basis of future manuscripts and funding applications.

	Year Quarter	2020		2021				2022	
		3	4	1	2	3	4	1	2
<b>Aim 1</b>	Spatial transcriptomics + sequencing	■	■						
	Differential gene analysis + cell annotation		■	■					
	Spatial mapping (bioinformatics)		■	■					
<b>Aim 2</b>	Development of bioinformatic interactome tools		■	■	■				
	Immunohistochemistry			■	■	■			
<b>Aim 3</b>	Cholangiocyte organoidogenesis				■	■	■		
	Immune cell-cholangiocyte organoid experiments							■	■
	Publish translational research article							■	■
	Publish methods paper							■	■

Completed Milestones: ■ Ongoing/future aims: ■

### 4. PUBLICATIONS & PRESENTATIONS

#### Publications

Chung BK, Øgaard J, Reims HM, Karlsen TH, Melum E. Spatial transcriptomics identifies enriched gene expression and cell types in human liver fibrosis. *Hepatol Commun.* 2022 Jun 20. doi: 10.1002/hep4.2001. Epub ahead of print. PMID: 35726350.

#### Presentations

Chung BK, Øgaard J, Reims HM, Karlsen TH, Melum E. Spatial transcriptomics identifies localization of enriched cell types in human liver fibrosis. *The Liver Meeting, American Association for the Study of Liver Diseases, 2021 Nov 12-15.*

### 5. LAY SUMMARY

We completed an analysis of total gene content from PSC liver samples using state-of-the-art tissue transcriptomics and observed a clear difference in anatomical gene expression that closely mapped to tissue regions classified as normal or fibrotic liver by conventional histology. Using gene expression detected in fibrotic liver regions, we then applied advanced bioinformatics (CIBERSORTx) to identify enriched cell types in fibrotic regions and confirmed our findings at the protein level by immunofluorescence staining. CIBERSORTx analysis revealed significantly greater numbers of mesenchymal cells, endothelial cells, monocytes, Kupffer cells and plasma cells in fibrotic liver regions suggesting that pathways in these cell types may be involved in progression of PSC. Taken together, these results indicate that tissue transcriptomics can

precisely distinguish gene expression from normal and diseased liver areas and that localized gene expression can be used to pinpoint the presence of specific cell types which may lead to the development of new treatments for PSC.