Single-cell Transcriptomic Profiling of MASH-HCC Development

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Background & Aim

Cellular-level insights into metabolic-associated steatohepatitis (MASH) and its progression to hepatocellular carcinoma (HCC) have been limited by bulk RNA-sequencing, which masks rare or tumor-relevant populations. Here, we applied single-cell transcriptomics to characterize liver cell-specific transcriptome changes in the GAN DIO-MASH-HCC mouse - a gold-standard translational model of MASH-driven HCC. The current study aimed to construct a comprehensive liver single-cell atlas of the healthy mouse, the GAN DIO-MASH and GAN DIO-MASH-HCC mouse models.

Methods

Male C57Bl/6J mice (n=3–4 per group) were fed chow (healthy lean mice) or the obesogenic GAN diet for 48 weeks (GAN DIO-MASH) or 68 weeks (GAN DIO-MASH-HCC). Paired tumor (HCC tumor) and adjacent non-tumorous (HCC non-tumor) samples was collected and dissociated into single cells and processed with the 10x Genomics GEM-X Flex 4-plex kit.

Conclusion

+ We obtained more than 200,000 cells to build a comprehensive liver single cell atlas
+ All major liver cell types were recovered in all study groups
+ Shifts in cell type distribution were observed during progression from CHOW to HCC tumor
+ Four distinct hepatocyte subpopulations were identified in HCC tumors
+ Tumor selective hepatocytes displayed enrichment of distinct pathways

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1 The GAN DIO-MASH and GAN DIO-MASH-HCC models

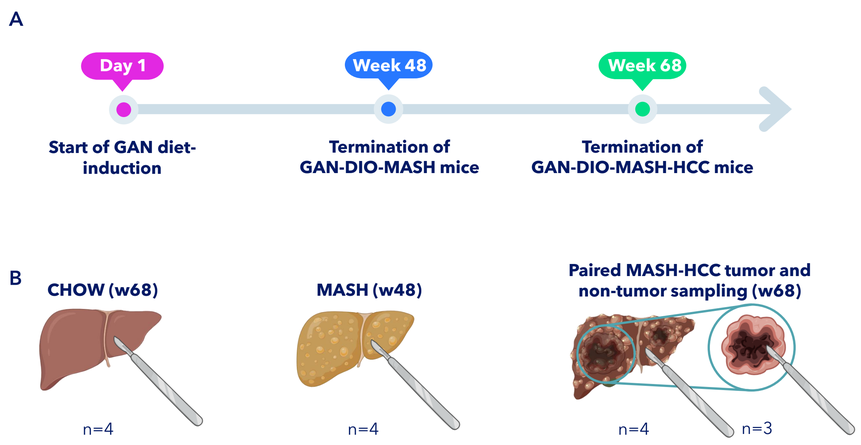

Figure 1. Study Overview of Mouse Liver Disease Models. (A) Timeline of GAN-diet induction for generation of the GAN DIO-MASH and GAN DIO-MASH-HCC mouse models. **(B)** Group overview: Age-matched lean chow-fed control mice (CHOW, w68, n=4), GAN DIO-MASH mice (w48, n=4), and GAN DIO-MASH-HCC mice (w68) with paired tumor (n=4) and tumor-adjacent samples (n=3). All collected samples were processed for single-cell RNA sequencing using the 10x Genomics GEM-X Flex Kit.

2 Hepatocellular atlas of the GAN DIO-MASH-HCC mouse

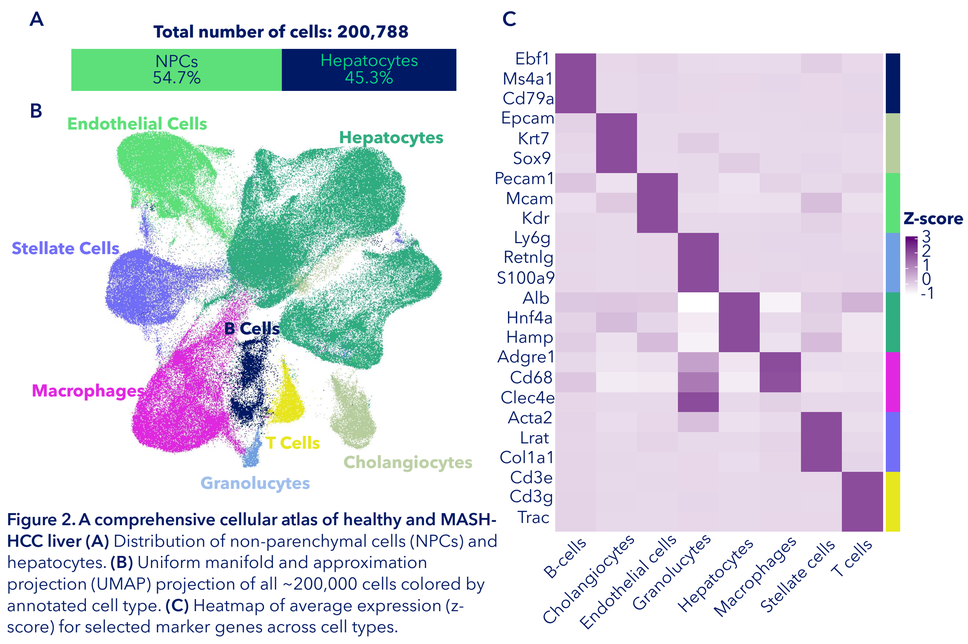

Figure 2. A comprehensive cellular atlas of healthy and MASH-HCC liver (A) Distribution of non-parenchymal cells (NPCs) and hepatocytes. **(B)** Uniform manifold and approximation projection (UMAP) projection of all ~200,000 cells colored by annotated cell type. **(C)** Heatmap of average expression (z-score) for selected marker genes across cell types.

3 Cell-type distribution during disease progression

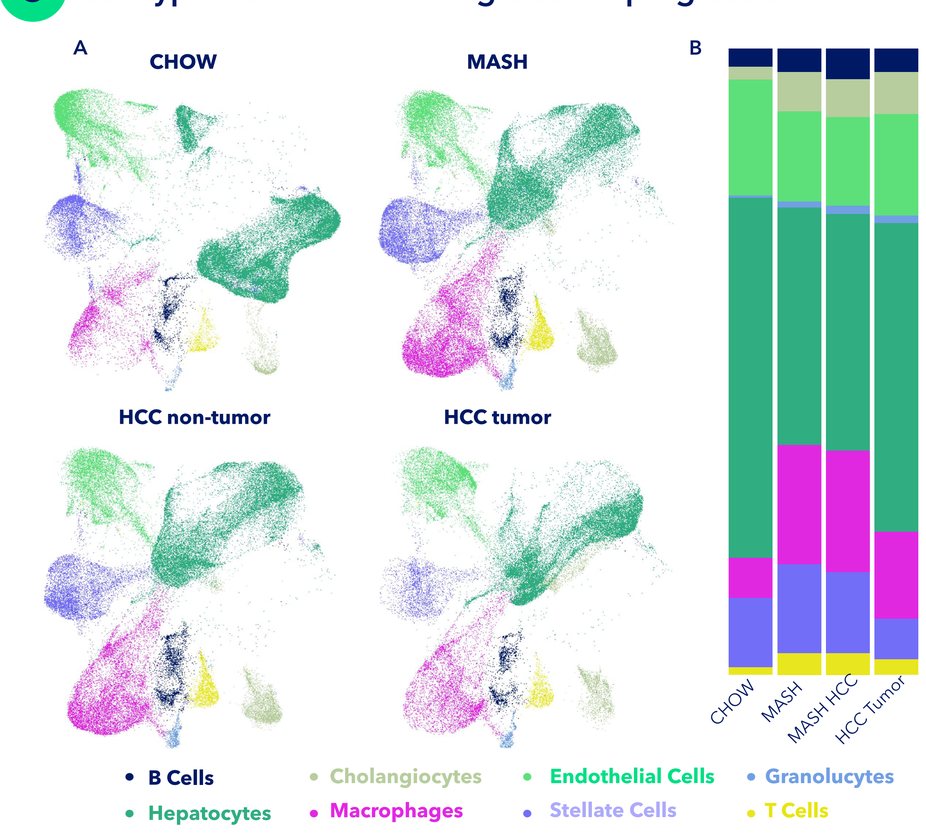

Figure 3. Single-Cell Landscape of HCC Progression. (A) UMAP projections of liver single-cell transcriptomes from CHOW, MASH, MASH-HCC and HCC tumor samples colored by annotated cell type to reveal changes in the cellular organization during disease progression. **(B)** Stacked bar plot summarizing relative abundance of cell types across the four conditions, with each bar normalized to 100% to illustrate compositional changes in the liver environment.

4 Hepatocyte subpopulations in MASH-HCC

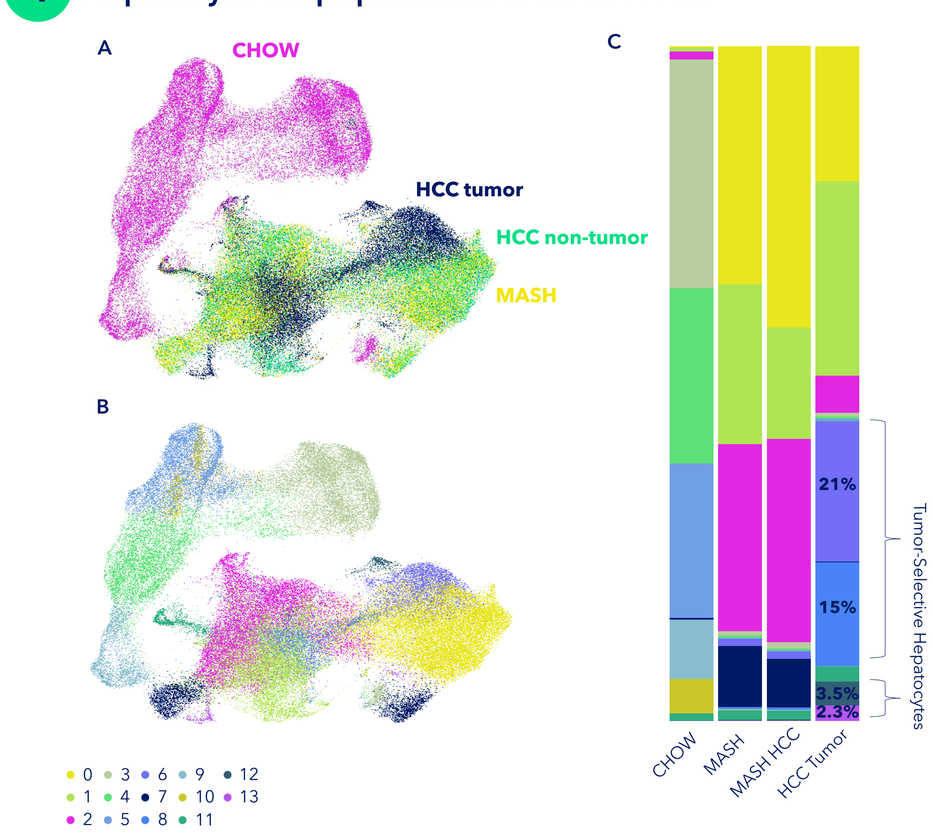

Figure 4. Hepatocyte subpopulations in MASH-HCC progression . (A) UMAP projection of all hepatocytes colored by group. **(B)** The same UMAP colored by hepatocyte subpopulations as determined by unsupervised clustering. **(C)** Stacked bar plot summarizing relative abundance (normalized to 100%) of hepatocyte subpopulations across all four conditions highlighting four tumor-selective hepatocyte populations.

5 Characteristics of Tumorous Hepatocytes

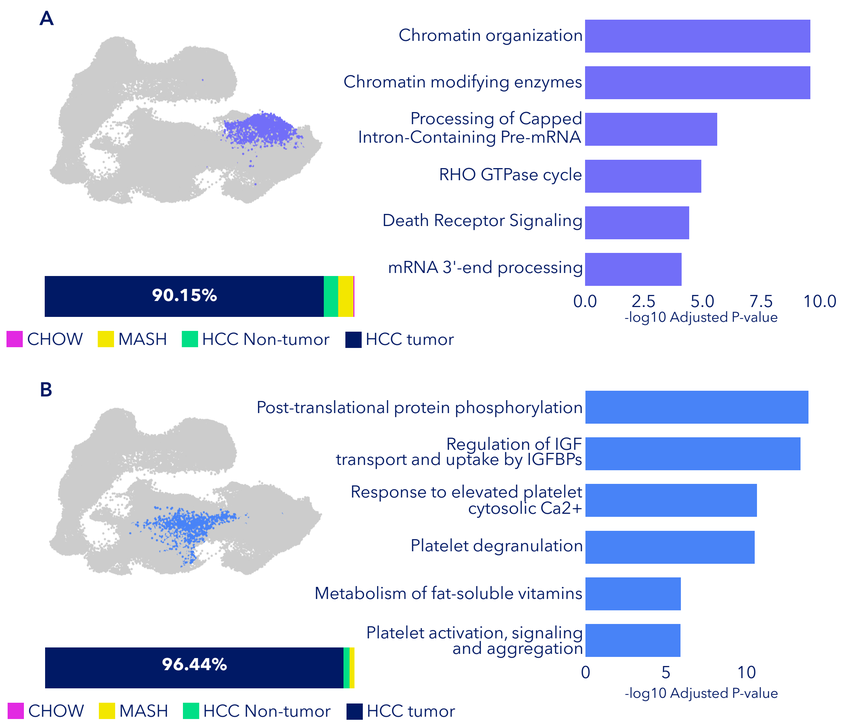

Figure 5. Functional Profiling of Tumor-Specific Hepatocyte Clusters. (A-D) Four panels each representing a tumor-selective hepatocyte sub population as identified in **(4)**. Each panel shows hepatocyte UMAP highlighting the subpopulation, composition bar plot showing the percentage of given cell populations deriving from each group, and Reactome pathway enrichment analysis of markers of given sub population.