Single-cell transcriptomics of intrahepatic cholangiocarcinoma (iCC) reveals novel tumor epithelial-stromal interactions

Richa John, Maria E. Monberg, Jaewon J. Lee, Mohamed M. Zaid, Bret M. Stephens, Naruhiko Ikoma, John Lowengrub, Eugene J. Koay, Paola A. Guerrero and Anirban Maitra.

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Introduction

Intrahepatic cholangiocarcinoma (iCCA), a rare form of liver cancer (0.3 – 6 for every 100,000 people) [1], has seen an increase in incidence and mortality in recent years [1]. Typically, patients diagnosed have a 5-year survival rate of 7-20% [2]. Thus, elucidating the molecular underpinnings of tumor progression are paramount. Here, single-cell RNA sequencing (scRNAseq) collected from six CCA patients was analyzed to discover ligand-receptor interactions between different cell types across various tissue compartments (central area of the tumor, tumor periphery and adjacent normal) in order to better understand the tumor progression of iCCA.

Materials & Methods

Liver resection samples for six patients were processed using 10x Genomics 5' RNA kits. Libraries were multiplexed and sequenced using an Illumina platform sequencer. Raw reads were subsequently aligned and converted to matrix files using CellRanger analysis pipelines, provided by 10x Genomics software. Downstream scRNAseq analysis was done using R packages Seurat [3], scMC [4], and CellChat [5].

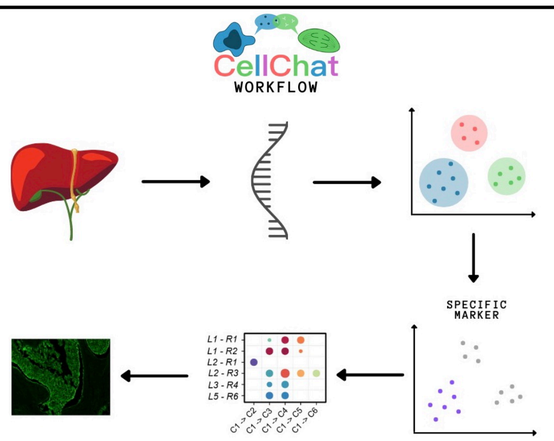

Fig. 1 Overview of workflow.
ScRNAseq data from six CCA patients were clustered according to cell type. Next, ligand-receptor interactions were predicted based on communication probability and assessed across three tissue compartments. Finally, the discovered interactions are validated by multiplex immunohistochemistry.

Results

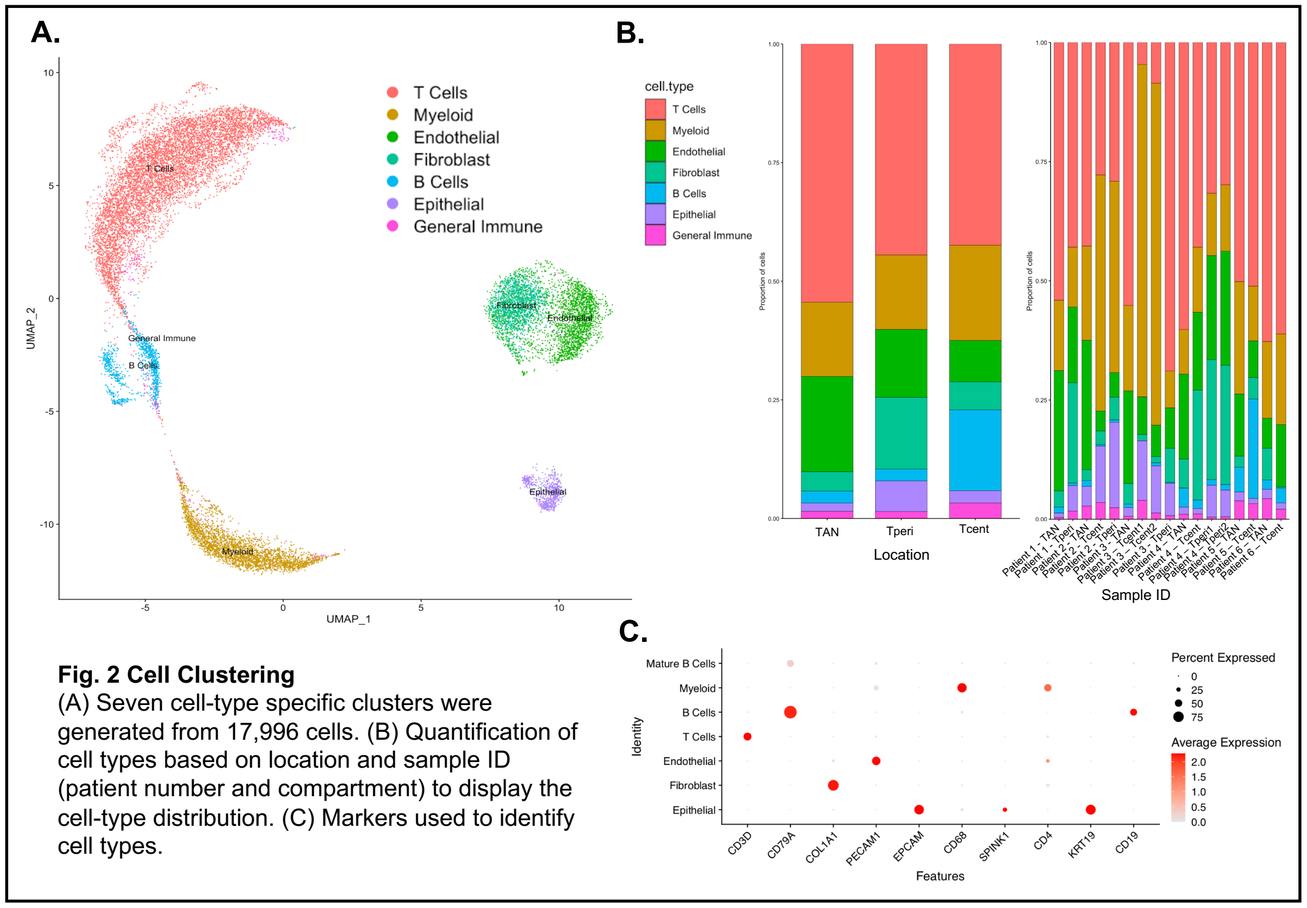

Fig. 2 Cell Clustering
(A) Seven cell-type specific clusters were generated from 17,996 cells. (B) Quantification of cell types based on location and sample ID (patient number and compartment) to display the cell-type distribution. (C) Markers used to identify cell types.

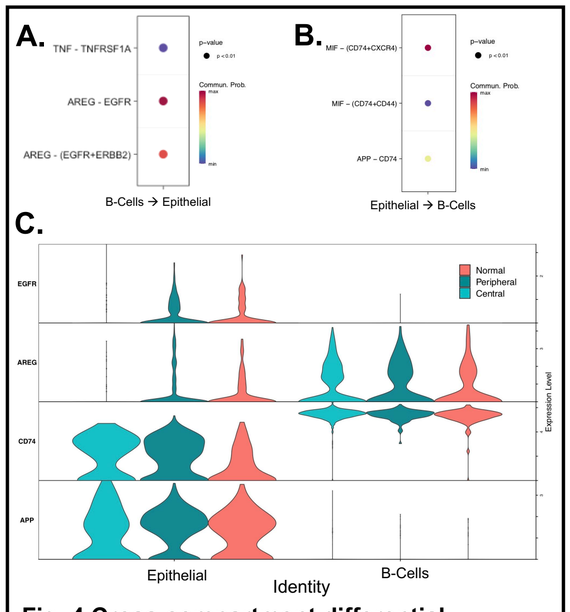

Fig. 4 Cross compartment differential expression of ligand-receptor interactions
(A) Specific ligand-receptor interactions of epithelial and B-cells. AREG-EGFR (B-cells to epithelial) and (B) APP-CD74 (epithelial to B-cells) are the two most significant interactions. (C) Expression of genes associated with these ligand-receptor pairs are shown cross-compartmentally, exhibiting regional exclusivity of gene expression and ligand-receptor cross-talk.

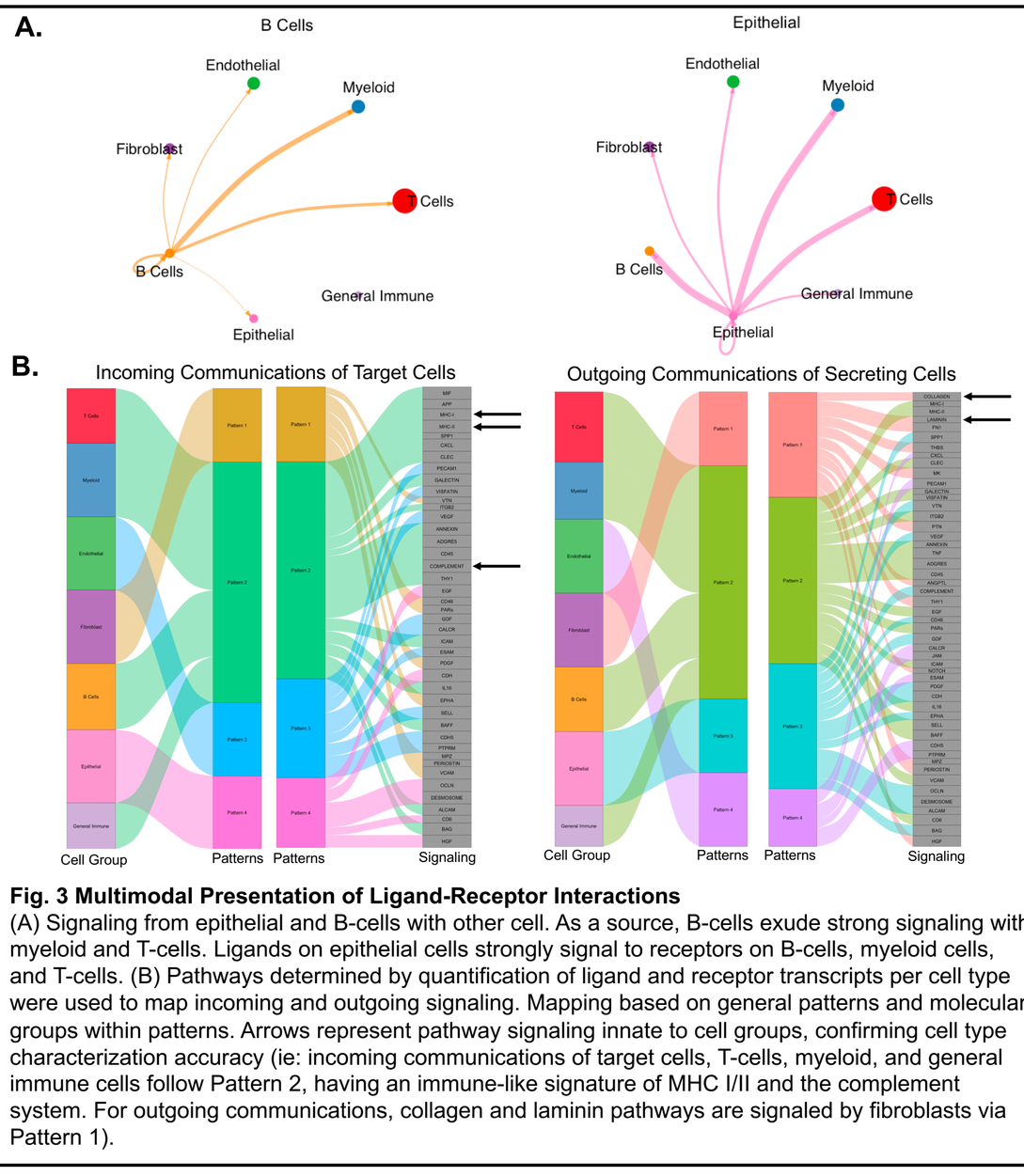

Fig. 3 Multimodal Presentation of Ligand-Receptor Interactions
(A) Signaling from epithelial and B-cells with other cell. As a source, B-cells exude strong signaling with myeloid and T-cells. Ligands on epithelial cells strongly signal to receptors on B-cells, myeloid cells, and T-cells. (B) Pathways determined by quantification of ligand and receptor transcripts per cell type were used to map incoming and outgoing signaling. Mapping based on general patterns and molecular groups within patterns. Arrows represent pathway signaling innate to cell groups, confirming cell type characterization accuracy (ie: incoming communications of target cells, T-cells, myeloid, and general immune cells follow Pattern 2, having an immune-like signature of MHC I/II and the complement system. For outgoing communications, collagen and laminin pathways are signaled by fibroblasts via Pattern 1).

Conclusions

In this study, we utilized CellChat to predict significant interactions among seven cell types present in the patient tissue samples derived from scRNAseq data. Interactions were examined across three tissue compartments to determine location-specific ligand-receptor interactions present. Moving forward, we will validate ligand-receptor pairs in tissue blocks by applying multiplex immunohistochemistry. By identifying important cell-to-cell interactions that potentially promote tumor progression, we can develop a strategy for targeted therapeutics against this deadly cancer, as a true application of precision-medicine approaches.

Acknowledgments

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References

1) Banales, J. M., et al. (2020). "Cholangiocarcinoma 2020: the next horizon in mechanisms and management." Nat Rev Gastroenterol Hepatol 17(9): 557-588.
2) Chan, K. M., et al. (2018). "Characterization of intrahepatic cholangiocarcinoma after curative resection: outcome, prognostic factor, and recurrence." BMC Gastroenterol 18(1): 180.
3) Stuart, T., et al. (2019). "Comprehensive Integration of Single-Cell Data." Cell 177(7): 1888-1902 e1821.
4) Zhang, L. and Q. Nie (2021). "scMC learns biological variation through the alignment of multiple single-cell genomics datasets." Genome Biol 22(1): 10.
5) Jin, S., et al. (2021). "Inference and analysis of cell-cell communication using CellChat." Nat Commun 12(1): 1088.