PROJECTEXVIVO

Dana-Farber Cancer Institute

Broad Institute with Microsoft

DEFINING TRANSCRIPTIONAL PHENOTYPES AND SPATIAL ORGANIZATION IN BILIARY TRACT CANCER

Andrew Navia[1], Sharon Bader[1,3], Akshaya Thoutam[1], Xiang Zhang[1,3], Michal Lipinski[1], Nicolas Fernandez[1], Tamara Aleksanyan[1], Jaspreet Ishar[1], Grace Joyner[3], Leigh Culnane[3], William Tan[3], Matthew Carnes[3], Lauren Brais[3], Elizabeth Andrews[3], Ewa Sicinska[3], Brian Wolpin[2,3], James Cleary[2,3], Sami Farhi[1], Peter Winter[1], Srivatsan Raghavan[1,2,3]

[1]Broad Institute of MIT and Harvard, [2]Harvard Medical School, [3]Dana-Farber Cancer Institute
Contact: Srivatsan_Raghavan@dfci.harvard.edu; ExVivo.BroadInstitute.org

Biliary tract cancers (BTC) are an aggressive family of malignancies characterized by significant heterogeneity and poor patient outcomes. While numerous efforts have characterized BTCs genomically, few studies have focused on the RNA classification and microenvironment structure of primary BTCs at single-cell resolution. Here, we present our work to identify conserved malignant cell RNA expression states and benchmark PDX model systems against their primary patient counterparts.

Methods: We performed probe-based single-cell RNA sequencing (scRNA-seq, 10X Genomics Flex) and single-cell spatial RNA-seq (StrataMap, Illumina; Xenium, 10X Genomics) on a cohort of BTC resection specimens including intra- and extra-hepatic cholangiocarcinoma and gallbladder carcinoma. A subset of these samples had matched PDX models that were also analyzed.

Results: Using 10X Flex we successfully captured a total of 232,631 patient cells across both malignant and non-malignant compartments. Using non-negative matrix factorization (NMF), we identified multiple conserved malignant cell RNA expression states, some of which were unique to specific disease subtypes (e.g., intra- versus extra-hepatic cholangiocarcinoma). Cross-correlation analysis comparing these single-cell programs to literature-curated gene sets demonstrated overlaps with major states described in prior bulk RNA-seq datasets but also revealed several new gene expression programs. These states and their corresponding spatial niches were also evaluated using StrataMap and Xenium.

Clinical and genomic features of the study cohort

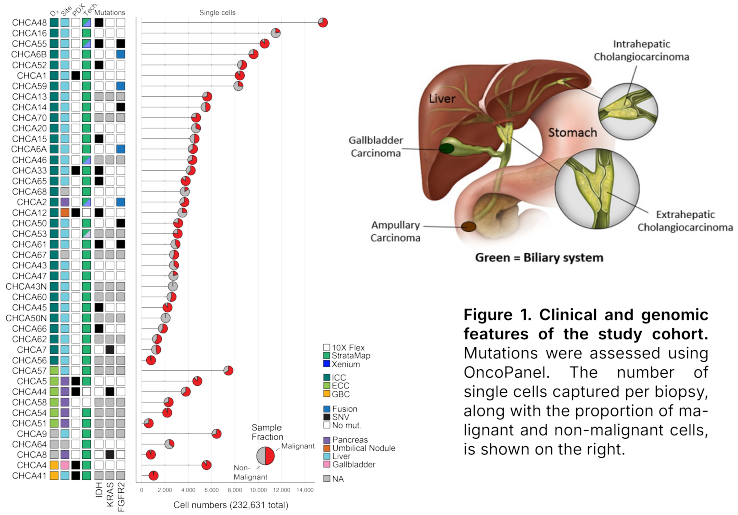


Figure 1. Clinical and genomic features of the study cohort. Mutations were assessed using OncoPanel. The number of single cells captured per biopsy, along with the proportion of malignant and non-malignant cells, is shown on the right.

Conclusions

We are continuing to interrogate this dataset to identify transcriptional programs associated with specific BTC genotypes. In future efforts, we plan to relate our dissociative scRNA-seq findings to spatial transcriptomic measurements across a broader set of clinical BTC tissue microarrays. We anticipate that these high-resolution maps of BTC tumors and paired patient models will provide a blueprint for understanding how BTC transcriptional states shape malignant cell behaviors and will uncover new approaches to therapeutically target this challenging set of cancers.

Funding sources

Illumina Catalyst Project, NCI/NIH, Dana-Farber Cancer Institute Philanthropic Funds, DFCI Hale Family Center for Pancreatic Cancer Research, Microsoft, Project Ex Vivo

10X Flex Data

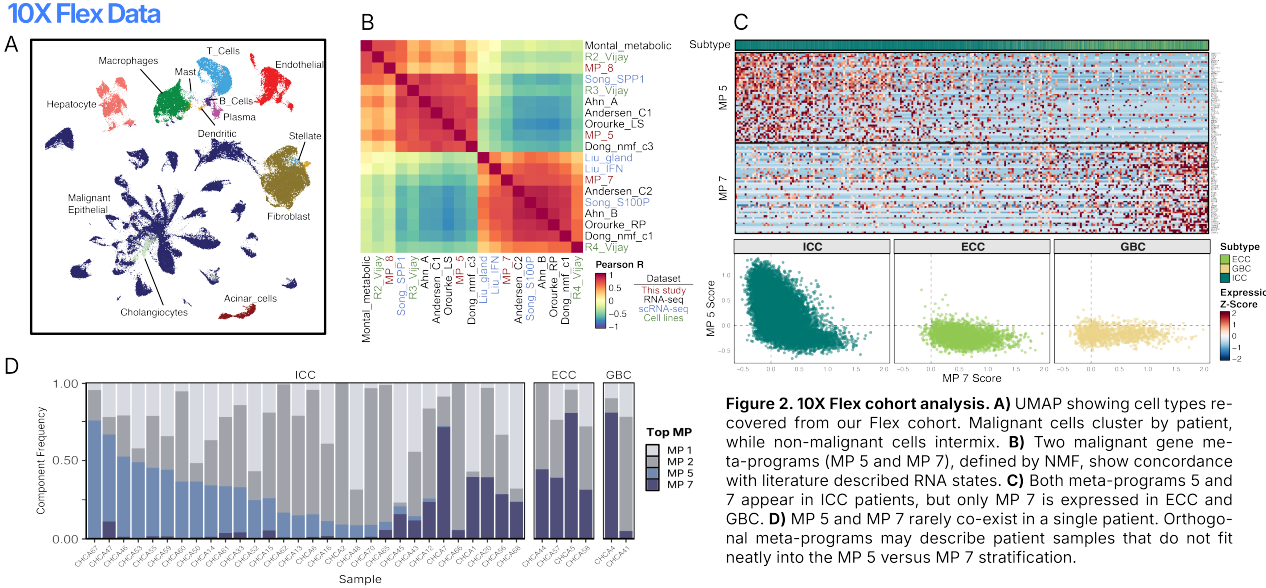


Figure 2. 10X Flex cohort analysis. A) UMAP showing cell types recovered from our Flex cohort. Malignant cells cluster by patient, while non-malignant cells intermix. **B)** Two malignant gene meta-programs (MP 5 and MP 7), defined by NMF, show concordance with literature described RNA states. **C)** Both meta-programs 5 and 7 appear in ICC patients, but only MP 7 is expressed in ECC and GBC. **D)** MP 5 and MP 7 rarely co-exist in a single patient. Orthogonal meta-programs may describe patient samples that do not fit neatly into the MP 5 versus MP 7 stratification.

Xenium Data

StrataMap Data

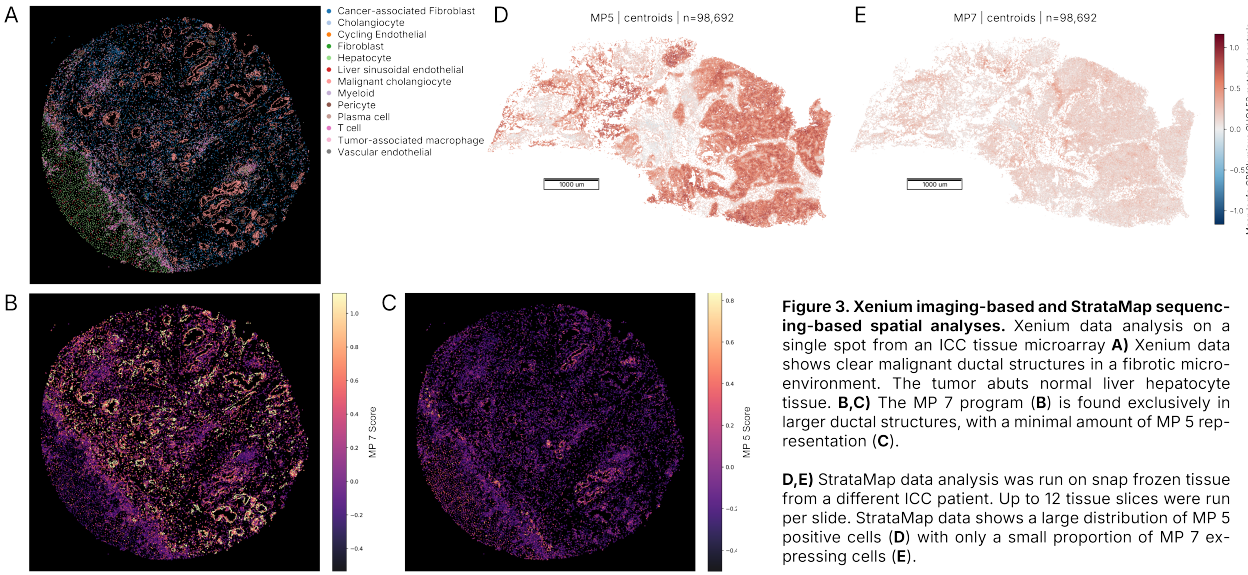


Figure 3. Xenium imaging-based and StrataMap sequencing-based spatial analyses. Xenium data analysis on a single spot from an ICC tissue microarray **A)** Xenium data shows clear malignant ductal structures in a fibrotic microenvironment. The tumor abuts normal liver hepatocyte tissue. **B,C)** The MP 7 program (**B**) is found exclusively in larger ductal structures, with a minimal amount of MP 5 representation (**C**).

D,E) StrataMap data analysis was run on snap frozen tissue from a different ICC patient. Up to 12 tissue slices were run per slide. StrataMap data shows a large distribution of MP 5 positive cells (**D**) with only a small proportion of MP 7 expressing cells (**E**).

Dana-Farber Cancer Institute · BROAD INSTITUTE · Microsoft · HARVARD MEDICAL SCHOOL