

**Paper 9. AN INTEGRIN-LAMININ GRADIENT DRIVES INFLAMMATION-TO-MALIGNANCY
TRANSITION IN DISTAL CHOLANGIOCARCINOMA**

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Conflicts of Interest:

J.C. received personal fees from Novartis outside the submitted work. V.C. obtained personal fees from Novartis during the conduct of the study. A.S.L. received grant funding from Novartis and NIH during the conduct of the study. The other authors have no relevant disclosures.

Background: Pancreatic ductal adenocarcinoma remains one of the deadliest human malignancies with 5-year overall survival <15%. Yet, its clinical mimic, distal cholangiocarcinoma (dCCA), remains understudied despite evidence linking chronic pancreatitis (CP) to biliary tract malignancy. The molecular mechanisms by which chronic pancreatic inflammation drives cholangiocarcinogenesis remain fundamentally unresolved, limiting diagnostic precision and therapeutic innovation.

Methods: We performed single-cell RNA sequencing on pancreatic/tumor specimens from healthy controls (n=3), CP patients (n=3), and dCCA patients (n=2). We profiled ~120,000 cells across 35 transcriptionally distinct clusters and delineated ductal cell heterogeneity through semi-supervised learning and pathway inference. We identified copy number variations (CNVs), validated malignant identity via KRAS mutation detection, and performed ligand-receptor modeling to decode cell-cell communication.

Results: We identified a previously undescribed ductal cell subpopulation (Ductal A) in dCCA displaying recurrent chromosomal amplifications (Chr 1, 2, 11, 12, 18) and KRAS G12C activating mutations (Figure 1). Critically, ITGA6—a gene mapped to the amplified chromosome 2 region—emerged as a key distinguishing feature of malignant ductal phenotypes. Ligand-receptor analysis revealed that ITGA6-LAMA4-LAMC2 axis signaling was uniquely amplified in dCCA compared to CP (Figure 1). Furthermore, cell surface glycoproteins and integrin receptors demonstrated a protein expression gradient: low in normal ductal cells, moderate in CP, and enriched in dCCA – revealing a molecular continuum from inflammation to malignancy.

Conclusions: These findings reveal a distinct fibroinflammatory molecular signature linking chronic pancreatitis to dCCA tumorigenesis through progressive integrin-laminin pathway activation. ITGA6 quantification may serve as a diagnostic biomarker, while the ITGA6-LAMA4-LAMC2 axis represents a novel therapeutic vulnerability in cholangiocarcinoma.

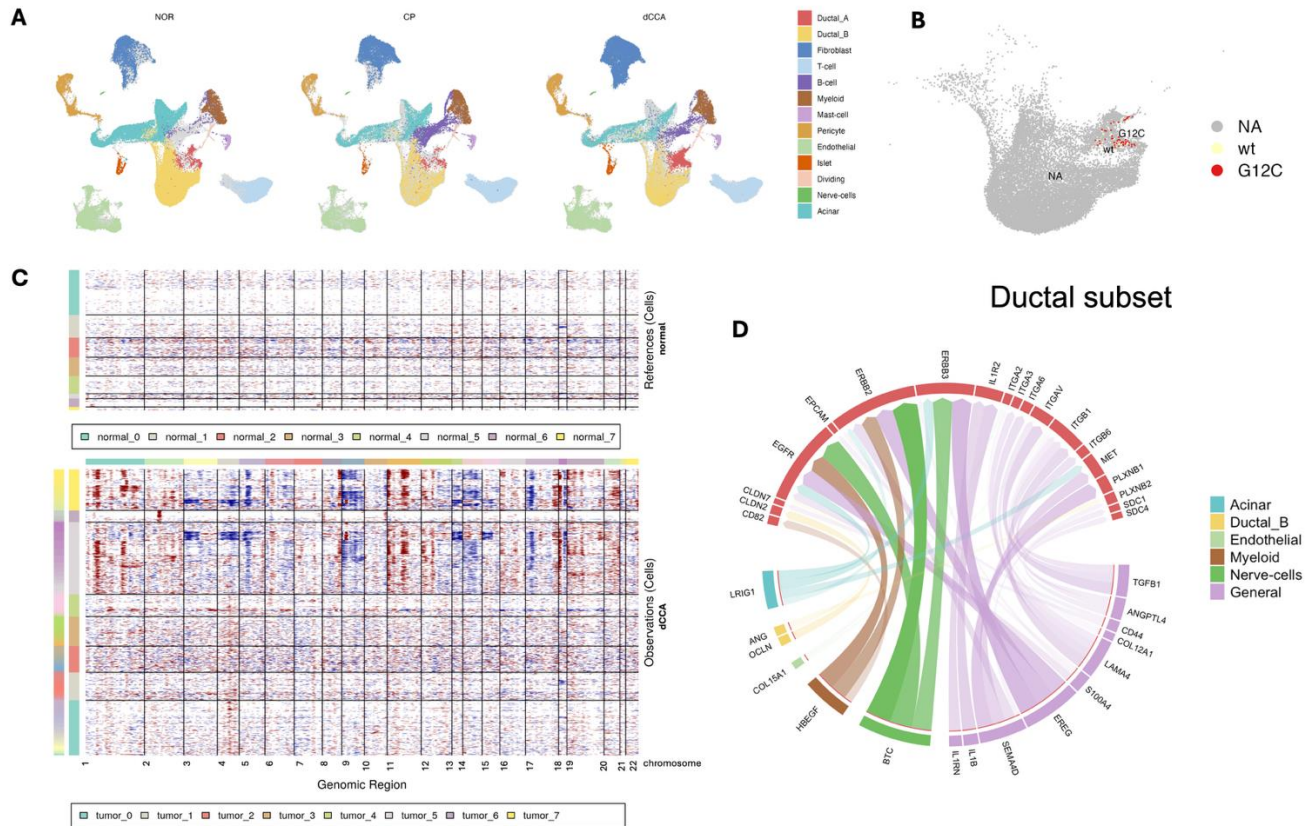


Figure 1. Single-Cell genomics analysis reveals a fibroinflammatory mechanism in the tumorigenesis of distal cholangiocarcinoma. **A.** UMAP visualization of single cells from normal (NOR; n=3), chronic pancreatitis (CP; n=3), and dCCA (n=2) specimens, where cells are colored by cell type. **B.** KRAS G12C mutation status projected onto the ductal cell subset. **C.** CNV inference for normal and dCCA ductal cell subsets, identifying novel aberrations in chromosomes 1, 2, 11, 12 and 18. **D.** Circos plot of receptor-ligand analysis and cell-to-cell communication highlights the cross-talk between laminins (e.g., LAMA4) and integrins in ductal A cells (receiving cells in red)