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Integrated RNA Sequencing, Whole-Genome Sequencing, and Spatial Profiling Reveal Distinct Tumor Microenvironment Architectures Across Anatomical Subtypes of Cholangiocarcinoma

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Background: Cholangiocarcinoma (CCA) encompasses three anatomically and biologically distinct subtypes— intrahepatic (iCCA), perihilar (pCCA), and distal (dCCA)—each with unique clinicopathological features, genomic landscapes and therapeutic vulnerabilities. We hypothesized that these subtypes harbor distinct tumor microenvironment (TME) architectures shaped by both shared and subtype specific genomic features.

Methods: We performed integrated multi-omic profiling of 56 treatment-naïve, resected specimens (iCCA, n = 24; pCCA, n = 9; dCCA, n = 17; normal liver or bile duct control, n = 6). Bulk RNA sequencing (RNA-seq) characterized global transcriptional programs, while whole-genome sequencing (WGS) catalogued somatic mutations, copy number alterations, and structural variants. High resolution targeted single-cell spatial transcriptomic profiling was performed using a customized CCA-focused panel. Differential expression, pathway enrichment, granular cell type and state annotation and spatial neighborhood analyses were conducted.

Results: RNA-seq revealed subtype-specific transcriptional programs; iCCA was enriched for B cell regulation and angiogenic signature; pCCA exhibited unique down regulation in TNF-alpha and NF-κB; whereas dCCA showed unique upregulation in coagulation/hemostasis and mesenchymal activation pathway. WGS confirmed established subtype-associated genomics alterations. Spatial profiling identified distinct immune architectures, including differences in tumor–immune interface density, cancer-associated fibroblast–rich stromal compartments, and patterns consistent with immune-excluded versus inflamed TMEs. Integration of genomic and spatial datasets suggests genotype-associated modulation of immune contexture.

Conclusions: We present a multi-omic dataset of the three major anatomical subtype of CCA. These findings demonstrate the existence of subtype-specific TME states and establish a framework for spatial biomarker discovery and genomically informed immunotherapeutic stratification.