Background Cholangiocarcinoma (CCA) is an aggressive malignancy of the biliary tract that carries an unfavorable prognosis. Recurrent, hotspot mutations in the IDH1 gene are found in 10–20% of CCAs and can be targeted with mutant IDH1 inhibitors, though objective responses leading to a reduction in tumor size are rare.1 2 Mutant IDH1 has neomorphic enzymatic activity that results in the production of the oncometabolite D-2-hydroxyglutarate (D-2-HG).3 D-2-HG promotes biliary tumor formation through cancer cell-intrinsic effects,4–6 but D-2-HG can also act as a paracrine factor released by IDH1-mutant cancer cells into the tumor microenvironment to promote tumor growth through non-cell intrinsic mechanisms.7–9 We have performed studies to determine the paracrine effects of D-2-HG on fibroblasts to further examine the CCA tumor microenvironment.

Methods To determine if fibroblasts are paracrine targets of D-2-HG in the CCA TME, we treated LX-2 hepatic stellate fibroblast cells with 0–50mM exogenous D-2-HG and utilized liquid chromatography-mass spectrometry to quantify the amount of intracellular D-2-HG. D-2-HG treated LX-2 fibroblasts and controls were then examined for changes in gene expression across 579 immune-related genes using the Nanostring platform. In partnership with Tempus, bulk RNA sequencing of IDH1-mutant (N=52) and wild type (N=403) CCA patient tumor samples was performed and CIBERSORT was used for deconvolution of gene expression data to define tumor-infiltrating immune cell populations.

Results Intracellular D-2-HG was increased in LX-2 cells treated with exogenous D-2-HG compared to controls (figure 1A). D-2-HG treated fibroblasts showed significant changes in immune-related gene expression with significant increases in expression of genes involved in immunometabolism, TLR signaling, and inflammasome signaling—as indicated by unsupervised hierarchical clustering (figure 1B). The most upregulated gene in D-2-HG-conditioned LX-2 fibroblasts is SPP1, which has been implicated in the recruitment and polarization of immunosuppressive M2 macrophages leading to decreased antitumor immunity.10–12 Interestingly, our analyses of resected human CCA samples showed that the IDH1-mutant CCA tumor immune microenvironment is characterized by an increase in M2 macrophages. Further study of how D-2-HG dysregulates fibroblast gene expression and affects tumor-infiltrating immune cell populations is warranted.

REFERENCES

Abstract 944 Figure 1 D-2-HG induces gene expression changes in fibroblasts. (A) Mass-spec analysis of intracellular D-2-HG levels in LX-2 fibroblasts treated with control or D-2-HG containing media. (B) Gene expression pattern for SPP1 in intracellular D-2-HG treated LX-2 fibroblasts as indicated by unsupervised hierarchical clustering. (C) Normalized mRNA expression for selected genes in LX-2 cells treated with control (black) or 50mM D-2-HG (red) containing media reveal upregulation of multiple genes that may alter the tumor immune microenvironment in CCA (*P<0.05, **P<0.01).

Abstract 944 Figure 2 The infiltrating immune cell populations in IDH1-mutant CCA. (A) RNA sequencing data from IDH1-mutant and wild-type CCA tumor samples was deconvoluted with CIBERSORT to define tumor-infiltrating immune cell populations. Cell populations with significant increases or decreases (Mann-Whitney U test, p<0.01) are plotted in a color spectrum to represent Log2FC in IDH1-mutant vs. wild-type tumors. (B) Box plot of the proportion of tumor-infiltrating immune cells identified as M2 macrophages in IDH1 wild-type (turquoise) and IDH1-mutant (gold) patient tumor samples (**P<0.001)
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Ethics Approval
This study was approved by the Johns Hopkins Hospital IRB: IRB approval number CR00023377.

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