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 0–50 mM 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 (figure 1C). We further identified that human IDH1-mutant CCA samples have a unique tumor immune microenvironment (figure 2A) and a significantly higher number of infiltrating M2 macrophages compared to wild-type controls (figure 2B).

Conclusions D-2-HG significantly alters gene expression in hepatic stellate cells, precursors to cancer-associated fibroblasts in CCA.9 The most upregulated gene in D-2-HG conditioned fibroblasts was 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


Ethics Approval This study was approved by the Johns Hopkins Hospital IRB: IRB approval number CR00023377.

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