FIBROBLASTS AS PARACRINE TARGETS OF THE ONCOMETABOLITE D-2-HG IN THE IDH1-MUTANT CHOLANGIOCARCINOMA TUMOR MICROENVIRONMENT

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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.^{7,8} 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.

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 patterns for 579 immune-related genes in the Nanostring Human Immunology V2 panel for LX-2 cells treated with control media (pink) or 50mM D-2-HG (gray) for 48 hours. D-2-HG treated cells have a distinct gene expression pattern 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)

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

1. Crispo F, Pietrafesa M, Condelli V, Maddalena F, Bruno G, Piscazzi A, *et al.* IDH1 targeting as a new potential option for intrahepatic cholangiocarcinoma treatment-current state and future perspectives. *Molecules* 2020;**25**. doi:10.3390/molecules25163754.
2. Abou-Alfa GK, Macarulla T, Javle MM, Kelley RK, Lubner SJ, Adeva J, *et al.* Ivosidenib in IDH1-mutant, chemotherapy-refractory cholangiocarcinoma (ClarIDHy): a multicentre, randomised, double-blind, placebo-controlled, phase 3 study. *Lancet Oncol* 2020;**21**:796–807.

3. Waitkus MS, Diplas BH, Yan H. Biological role and therapeutic potential of IDH mutations in cancer. *Cancer Cell* 2018;**34**:186–195.
4. Du X, Hu H. The roles of 2-Hydroxyglutarate. *Front Cell Dev Biol* 2021;**9**:651317.
5. Ma S, Jiang B, Deng W, Gu Z-K, Wu F-Z, Li T, et al. D-2-hydroxyglutarate is essential for maintaining oncogenic property of mutant IDH-containing cancer cells but dispensable for cell growth. *Oncotarget* 2015;**6**:8606–8620.
6. Saha SK, Parachoniak CA, Bardeesy N. IDH mutations in liver cell plasticity and biliary cancer. *Cell Cycle* 2014;**13**:3176–3182.
7. Böttcher M, Mougiakakos D. Immunometabolic regulation of anti-tumor T-cell responses by the oncometabolite d-2-Hydroxyglutarate. *Immunometabolism* 2019;**1**. doi:10.20900/immunometab20190007.
8. Friedrich M, Bunse L, Wick W, Platten M. Perspectives of immunotherapy in isocitrate dehydrogenase-mutant gliomas. *Curr Opin Oncol* 2018;**30**:368–374.
9. Ma L, Wang L, Khatib SA, Chang C-W, Heinrich S, Dominguez DA, et al. Single-cell atlas of tumor cell evolution in response to therapy in hepatocellular carcinoma and intrahepatic cholangiocarcinoma. *J Hepatol* 2021. doi:10.1016/j.jhep.2021.06.028
10. Zhang Y, Du W, Chen Z, Xiang C. Upregulation of PD-L1 by SPP1 mediates macrophage polarization and facilitates immune escape in lung adenocarcinoma. *Exp Cell Res* 2017;**359**:449–457.
11. Psallidas I, Stathopoulos GT, Maniatis NA, Magkouta S, Moschos C, Karabela SP, et al. Secreted phosphoprotein-1 directly provokes vascular leakage to foster malignant pleural effusion. *Oncogene* 2013;**32**:528–535.
12. Wei J, Marisetty A, Schrand B, Gabrusiewicz K, Hashimoto Y, Ott M, et al. Osteopontin mediates glioblastoma-associated macrophage infiltration and is a potential therapeutic target. *J Clin Invest* 2019;**129**:137–149.

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

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