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BAP1 loss confers ferroptosis resistance to cholangiocarcinoma via TLCD1-mediated mitochondrial membrane phospholipid remodeling

Yu Zhao¹, Peyton Classon¹, Danielle M. Carlson¹, Jayla T. Millender¹, Amro M. Abdelrahman³, Irene K. Yan², Sumera I. Ilyas¹, Rory L. Smoot³, Gregory J. Gores¹, Tushar C. Patel², Mark J. Truty³, Davide Povero^{1,4}

¹Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA; ²Division of Hepatology and Liver Transplant, Mayo Clinic, Jacksonville, FL, USA; ³Department of Surgery, Division of Hepatobiliary and Pancreatic Surgery, Mayo Clinic, Rochester, MN, USA; ⁴Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA

Background: Cholangiocarcinoma (CCA) is a deadly cancer of the hepatic bile duct epithelial cells. Loss-of-function mutations of epigenetic regulator ubiquitin C-terminal hydrolase deubiquitinase BRCA1-associated protein 1 (BAP1) occur in 26-32% of human CCA and are associated with worse prognosis and resistance to cytotoxic therapy. The goal of this study is to identify how BAP1 loss promotes cell death evasion through metabolic rewiring.

Materials and methods: Isogenic wild-type and BAP1-deficient human and murine CCA cell lines were generated with shRNA/siRNA or CRISPR/Cas9 and were used to assess ferroptosis sensitivity by treatment with ferroptosis inducers. *In vitro*, ferroptosis was assessed by phospholipid peroxidation (C-11BODIPY), mitochondrial phospholipid peroxidation (MitoPeDPP, MitoCLOx), iron overload (MitoFerro green) and cell death (CellTiter-Glo). Phospholipidomics and proteomics were performed in isogenic cells by LC-MS/MS. Syngeneic liver orthotopic CCA murine models and patient-derived xenografts (PDX) were used for *in vivo* pre-clinical studies.

Results: Our findings indicate that BAP1 loss confers CCA ferroptosis resistance. BAP1 loss protects CCA against mitochondrial iron overload, ROS accumulation, and phospholipid peroxidation. BAP1 loss protects from mitochondrial phospholipid peroxidation and cardiolipin oxidation. Unbiased phospholipidomic profiling revealed that BAP1 loss remodels membrane phospholipid composition, enriching for ferroptosis blocking monounsaturated phospholipids (MUFA-PLs) at the expense of ferroptosis-inducing polyunsaturated (PUFA-PLs). We found that BAP1 loss upregulates TLCD1, a phosphatidylethanolamine acyltransferase that incorporates MUFAs into membrane phospholipids, thereby stabilizing membranes against peroxidation. TLCD1 knockdown in ferroptosis-resistant BAP1-deficient CCA cells, restored ferroptosis sensitivity. Notably, TLCD1 is significantly upregulated in human BAP1-mutant CCA as compared to BAP1-expressing tumors. While not a mitochondrial protein, TLCD1 localizes in the ER and by the mitochondrial outer membrane, as demonstrated by our immunofluorescence analysis in human CCA cells. From a therapeutic perspective, we developed EpCAM (adhesion molecule highly expressed in CCA cells)-aptamer coated lactosomes packaged with siRNA against glutathione peroxidase 4 (GPX4), as a proof-of-concept strategy to safely induce ferroptosis selectively in CCA *in vitro* and *in vivo*. Administration of siGPX4-lactosomes in pre-clinical syngeneic liver orthotopic CCA murine models demonstrated a favorable safety profile, achieved robust intratumoral GPX4 knockdown (>50%), exhibited optimal biodistribution, induced significant tumor lipid peroxidation, and resulted in reduced tumor burden. Lastly, ferroptosis inducer JKE-1674, combined with gemcitabine/cisplatin (Gem/Cis) significantly blocked tumor growth of Gem/Cis-resistant patient-derived xenografts.

Conclusions: Our findings reveal that BAP1 loss reprograms mitochondrial phospholipid metabolism thus conferring CCA resistance to ferroptosis, identifying TLCD1 as a targetable metabolic vulnerability in BAP1-mutant CCA. Additionally, our findings support ferroptosis inducing compounds as promising therapeutic approaches for CCA.