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

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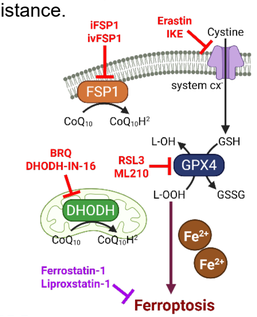
BACKGROUND

Cholangiocarcinoma (CCA) is a highly lethal epithelial cell malignancy of the biliary tract. CCA is characterized by a desmoplastic and immunosuppressive tumor microenvironment. CCA overall survival is <10% and only limited treatment options are available. Incidence and mortality are rising worldwide.

BAP1 (Ubiquitin C-terminal hydrolase deubiquitinase)

- Tumor suppressor and deubiquitinase
- Mutated in up to 32% of human CCAs
- Nuclear epigenetic regulator (H2AK119ub1 DUB)
- Cytosolic regulator cellular metabolism and cell death

Ferroptosis is an iron-catalyzed cell death driven by phospholipid peroxidation. Cancer metabolic reprogramming and genetic aberration may confer ferroptosis resistance.



AIM

The **goal** of this study is to identify how BAP1 loss promotes cell death evasion through metabolic reprogramming. This work may identify novel therapeutic opportunities for BAP1-mutant CCA.

METHODS

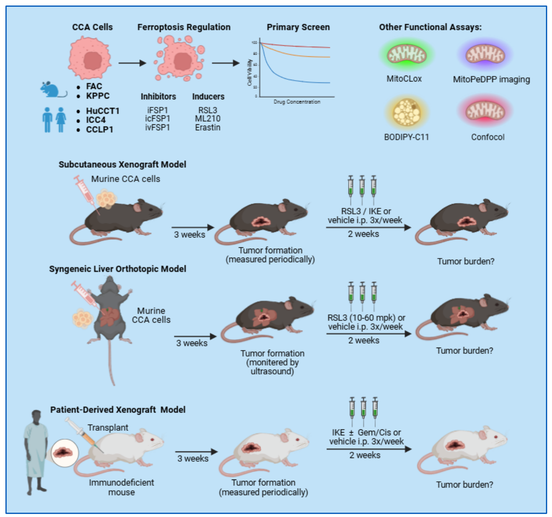


Figure 1. *In vitro* drug screening identified ferroptosis-sensitive and ferroptosis resistant CCA cells.

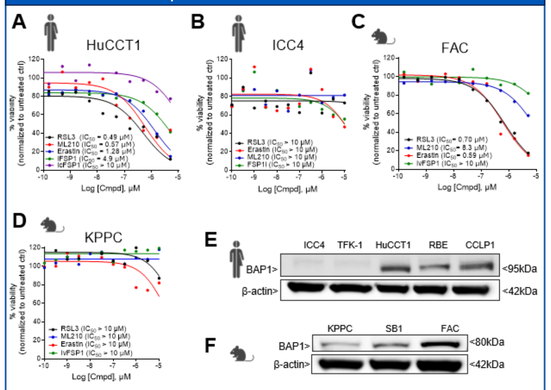


Figure 1. (**A-B**) Dose–response curves of human CCA cell lines (HuCCT1 and ICC4) treated with ferroptosis inducers. (**C-D**) Dose–response curves of murine CCA cell lines (FAC and KPPC) treated with ferroptosis inducers. Immunoblot analysis of BAP1 protein expression in (**E**) human CCA cell lines and (**F**) murine CCA cell lines. β-actin was used as loading control.

Figure 2. BAP1 loss confers CCA resistance to ferroptosis

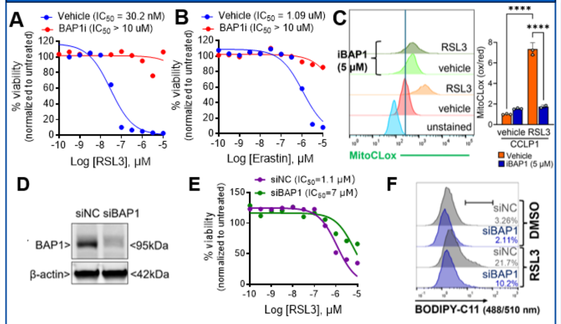


Figure 2. **(A–B)** Dose–response curves of RSL3 and Erastin in CCLP1 cells treated ±BAP1 inhibitor (1μM). **(C)** Flow cytometry for mitochondrial lipid peroxidation probe MitoCLox in CCLP1 cells ± RSL3 and BAP1 inhibitor. **(D)** Immunoblot of BAP1 in siNC vs. siBAP1 cells. **(E)** Dose–response curve of RSL3 in siNC vs. siBAP1 CCLP1 cells. **(F)** Flow cytometry of C11-BODIPY lipid peroxidation assay in siNC and siBAP1 cells ± RSL3.

Figure 3. BAP1 loss protects CCA from mitochondrial lipid peroxidation

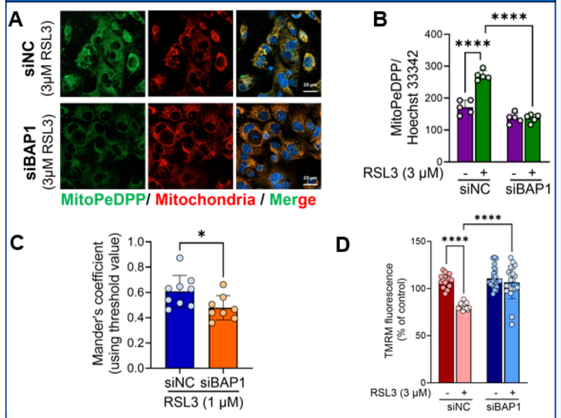
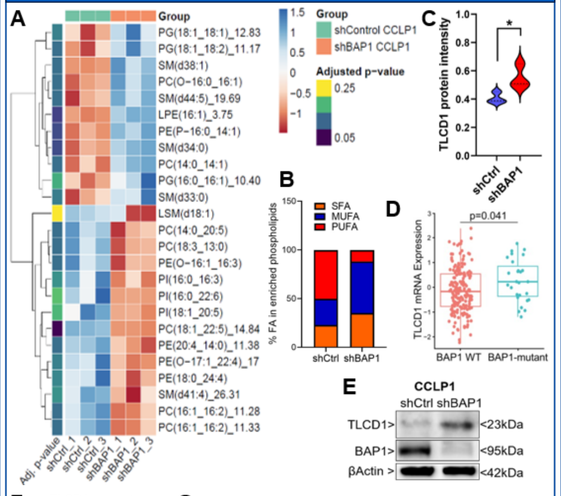


Figure 3. **(A-B)** Representative confocal images and quantification of mitochondrial lipid peroxidation probe MitoPeDPP (green) and mitochondria (red) in CCLP1 ±RSL3. **(C)** Colocalization of C11-BODIPY and Mitotracker assessed by Mander's coefficient in siNC vs. siBAP1 CCLP1 cells. **(D)** Mitochondrial membrane potential measured by TMRM in siNC vs. siBAP1 CCLP1 ± RSL3.

Figure 4. BAP1 loss remodels membrane phospholipid composition via TLCD1 activation.



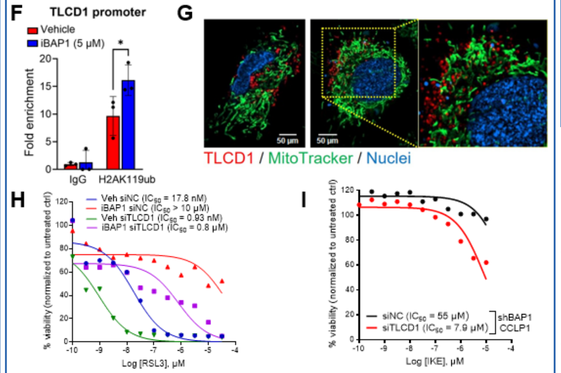


Figure 4. **(A–B)** Phospholipidomics heatmap and fatty acid-PL enrichment in shControl vs. shBAP1 CCLP1 cells. **(C)** Proteomics analysis identified lysophosphatidylethanolamine acyltransferase TLCD1 enrichment in shBAP1 CCLP1 cells. **(D)** TLCD1 RNA expression in WT and BAP1-mutant human CCA subjects (Mayo Clinic cohort, n=222 subjects). **(E)** TLCD1 immunoblot in shControl and shBAP1 CCLP1 cells. **(F)** ChIP–PCR for H2AK119ub1 at TLCD1 locus showing increased promoter occupancy upon BAP1 loss. **(G)** Confocal images of TLCD1 and Mitotracker staining. **(H–I)** Dose–response curves of RSL3 and IKE in BAP1-deficient CCLP1 cells ± siTLCD1.

Figure 5. Ferroptosis reduces CCA tumor burden and induces lipid peroxidation in murine and human CCA

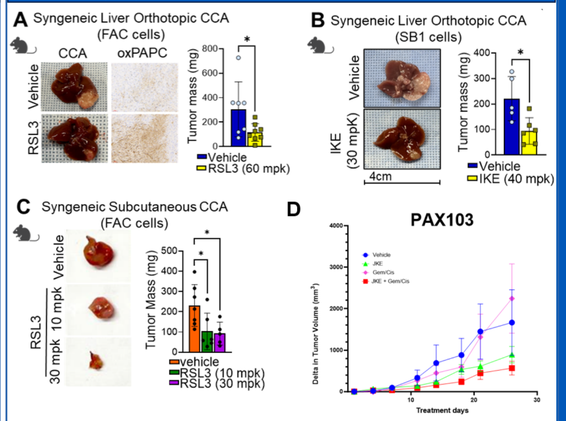


Figure 5. **(A–B)** Ferroptosis inducer RSL3 or IKE reduces tumor growth in two syngeneic orthotopic CCA cell liver implantation models (FAC and SB1 cells). **(C)** Ferroptosis suppresses tumor growth in syngeneic subcutaneous CCA models. **(D)** Ferroptosis inducer JKE-1674 (20 mpk) alone or with gemcitabine and cisplatin (Gem/Cis) reduces tumor burden of CCA patient-derived xenografts (PDXs). Ferroptosis inducers were administered intraperitoneally, intratumor or orally. mpk=mg/Kg.

CONCLUSIONS:

- BAP1 loss confers CCA resistance to ferroptosis, especially via protection against mitochondrial lipid peroxidation.
- BAP1 loss remodels membrane phospholipid composition via TLCD1 by enriching for ferroptosis-blocking MUFA-PLs at the expense of ferroptosis-inducing PUFA-PLs.
- *In vivo* ferroptosis reduces tumor growth in tumor-bearing murine CCA models.
- Ferroptosis inducer JKE-1674 blocks tumor growth and augments response to standard-of-care chemotherapy (Gem/Cis) in CCA PDX resistant to Gem/Cis.

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