**Background**

Targeting mitochondrial complexes is emerging as an effective chemotherapy strategy, prompting the urgency to better understand drug resistance to mitochondrial complex inhibitors. Here we report that intrinsic lipid metabolism contributes to resistance when targeting mitochondrial complex I in pancreatic ductal adenocarcinoma (PDAC). Our data indicates that induction of mitochondrial- and lipid- reactive oxidative species (ROS) is critical for complex I inhibition induced cell death. Lipidomic analysis revealed an abundance of ether-formed mono-unsaturated fatty acids (MUFAs), in cells resistant to complex I inhibition, is an essential fuel for ROS scavenging. Blocking ether-MUFAs by knocking out enzymes responsible for ether-formed phospholipids generation in peroxisome, sensitized resistant cells. Together, our findings uncovered a novel adaptive mechanism dependent on ether-lipids metabolism based on the peroxisome-mitochondria network that is responsible for PDAC resistance to OXPHOS inhibition, providing the rationale for combinatorial strategies to target mitochondria in PDAC.

**Result 1: Sensitive & Resistant response to mitochondrial Cl inhibitor in PDACs**

![Sensitive and Resistant response to mitochondrial Cl inhibitor in PDACs](image)

**Result 2: Mitochondrial ROS scavenging contributes to PDAC resistance**

![Mitochondrial ROS scavenging contributes to PDAC resistance](image)

(A) Lipidomics analysis showed more ether modified MUFAs in resistant lines than in sensitive lines. Mitochondrial ROS (B) and cell death (C) detection upon 3-days treatment of 10nM IACS-010759 in Ether generation deficiency by blocking GNPAT signaling. (D) Xenograft tumor growth of sg-TRL/sgGNPAT with or without 5mpk IACS-010759. Tumor volume was measured at the days indicated. (E) Cell death events in sgCTRL, sgGNPAT PDAC with ether-linked MUFAS (C16:18:1 PC). *P<0.05, **P<0.01, ***P<0.001.

**Result 3: Ether-lipid metabolism is crucial for PDAC resistance**

![Ether-lipid metabolism is crucial for PDAC resistance](image)

Sensitive (A) and Resistant (B) PATC lines were treated with 10nM IACS-010759 in 3 days. Mitochondrial ROS were detected by MitoSOX Dye. (C) H2O2 indicator showed ROS production with 10nM IACS-010759 in indicated time. Anti-TOMM20 reflected mitochondrial location. Scale bar, 10um. Mitochondrial-ROS inducer MitoPQ (C) and scavenger miTOQ(D) and SOD2 deficiency (E) affect PDAC resistance.

**Hypothesis model**

![Hypothesis model](image)

Cartoon working model. Ether linked- MUFAs generated through peroxisome and mitochondria contribute to complex I inhibition by scavenging mitochondrial- and lipid-ROS.

**References**