Targeting the fatty acid oxidation in combination with chemotherapy induces complete pancreatic cancer regression *in vivo*

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Background: Pancreatic Ductal AdenoCarcinoma (PDAC) represents one of the deadliest human cancers, with a 5-year relative survival rate of only 9% (https://ourworldindata.org/cancer). This is due to its late diagnosis, and lack of response to current treatments. Recently, our group demonstrated that **mitochondrial respiration (OXPHOS)** is an efficient target in a subset of patients with PDAC (Masoud *et al.*, 2020). Moreover, our current project focuses on a novel vulnerability of this mortal cancer, the **Fatty Acid Oxidation (FAO).**

OBJECTIVES

I. Evaluate the therapeutic potential of targeting mitochondrial FAO in PDAC;
II. Investigate the mechanisms behind the response to FAO targeting in combination with chemotherapy.

METHODS

Biological samples: Primary human PDAC cells (PaCaOmics cohort).

1. Mitochondrial metabolism analysis (Seahorse oxygraph)

Mito Fuel Flex Test: Rate of oxidation of glucose, glutamine, and fatty acids (by measuring Oxygen Consumption Rate, OCR)

2. Targeting FAO in combination with chemotherapy

Cell viability assays | Patient Derived Xenografts (PDX)

3. Tissular, cellular and molecular investigation

Histo/immunohisto-chemistry *ex vivo* | Metabolic (Seahorse) and cell death assays (flow cytometry) | Transcriptomic analysis (RNA-seq and RT-qPCR)

RESULTS

1. Mitochondrial respiration of primary PDAC cells is dependent on the FAO pathway

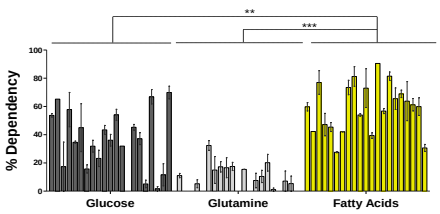

Fig 1. We used the specific inhibitors UK5099, BPTES, and Etomoxir, to determine OXPHOS dependency on glycolysis, glutaminolysis, and Fatty Acid Oxidation (FAO), respectively. The assay was performed in 21 primary PDAC cells (each bar represents a patient).

2. PDAC cells exhibit different sensitivities to FAO inhibitors (High vs. Low responders) and Perhexiline plus Gemcitabine is synergistic in High responders cells

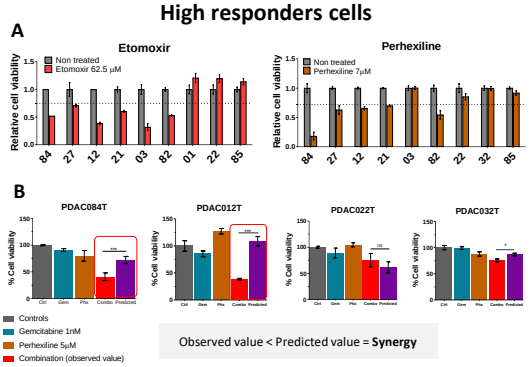

Fig 2. (A) We treated PDAC cells for 72 h with Etomoxir or Perhexiline, that inhibit the Carnitine Palmitoyltransferase 1 (CPT1), a critical fatty acid transporter into mitochondria. We identified "High responders" or "Low responders" cells i.e. by showing a relative cell viability below or above the mean. **(B)** Four PDAC cells were treated for 72 h with Perhexiline, Gemcitabine, or the combination.

RESULTS

3. Targeting FAO with Perhexiline in combination with Gemcitabine induces complete PDAC regression in a High responder xenograft

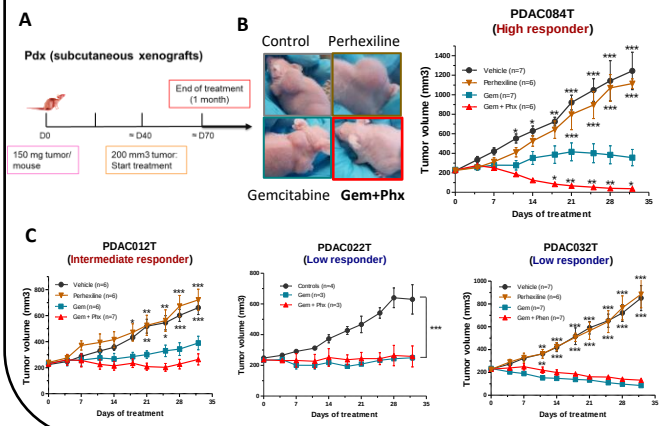

Fig 3. (A) Schematic protocol. **(B)** High responder PDAC xenograft. Photos of representative tumor-bearing mice (left), and tumor growth curve during one month treatment with Perhexiline, Gemcitabine, or the combination (right). **(C)** Tumor growth curves in intermediate and low responder xenografts.

4. Perhexiline plus Gemcitabine effectively suppress PDAC by inducing apoptosis and energetic stress

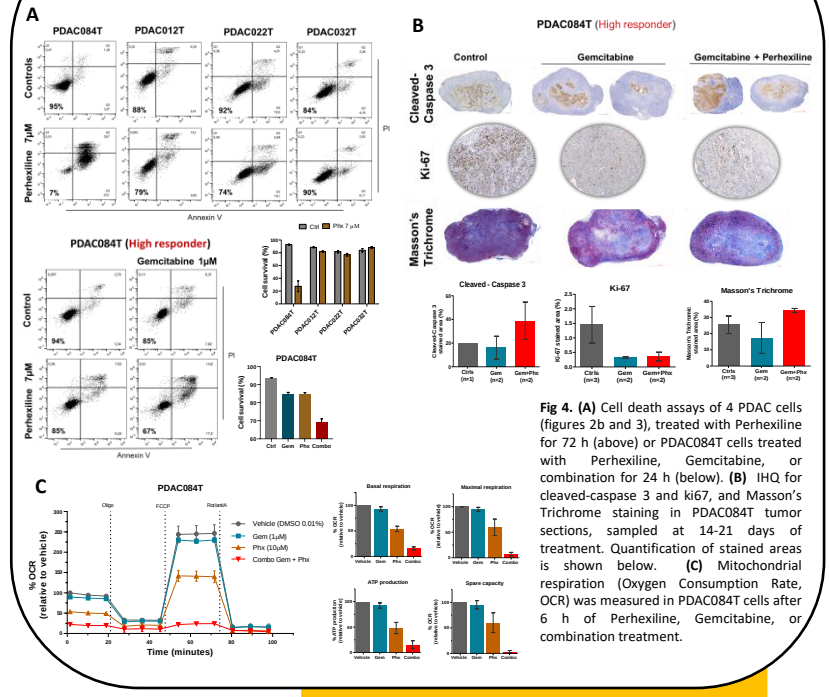

Fig 4. (A) Cell death assays of 4 PDAC cells (figures 2b and 3), treated with Perhexiline for 72 h (above) or PDAC084T cells treated with Perhexiline, Gemcitabine, or combination for 24 h (below). **(B)** IHQ for cleaved-caspase 3 and ki67, and Masson's Trichrome staining in PDAC084T tumor sections, sampled at 14-21 days of treatment. Quantification of stained areas is shown below. **(C)** Mitochondrial respiration (Oxygen Consumption Rate, OCR) was measured in PDAC084T cells after 6 h of Perhexiline, Gemcitabine, or combination treatment.

5. CPT1C as a key actor in the response to FAO targeting in PDAC

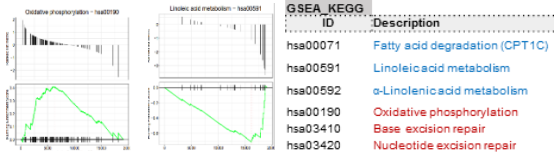

Fig 5. Downregulated (blue) and upregulated (red) pathways in the High responder cells to Perhexiline in comparison with Low responder cells.

CONCLUSIONS

FAO is a novel vulnerability of PDAC, and its targeting is a promising strategy to treat this cancer. This work provides the mechanism of cooperation between Perhexiline and Gemcitabine, and could lead to identify biomarkers to predict the response of patients to FAO targeting.

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