Lipid Metabolism supports the tumorigenic potential of Pancreatic Cancer Stem Cells

Courtois S., Bokil A., Santander-Badules H., Royo-García A. and Sancho P.

Instituto de Investigación Sanitaria de Aragón (IIS Aragón), Zaragoza, Spain

Introduction

- **Pancreatic ductal adenocarcinoma (PDAC)** is one of the deadliest tumours nowadays, partly due to the intrinsic aggressiveness and chemoresistance of resident subpopulations of cancer cells, the **Cancer Stem Cells (CSCs).** *These cells bear stemness-related properties and are responsible for tumour relapse and metastasis, representing a crucial target for long-term successful chemotherapeutic intervention*.
- In contrast to differentiated tumour cells, pancreatic CSCs are highly dependent on **mitochondrial oxidative phosphorylation** (OXPHOS, Sancho et al, Cell Metabolism 2015). This metabolism confers them with the ability of using a wider range of substrates able to feed the TCA cycle, including Fatty Acids (FA). Interestingly, **increased lipid scavenging and aberrant FA metabolism have been linked to pancreatic tumour progression and poor prognosis in PDAC patients.**

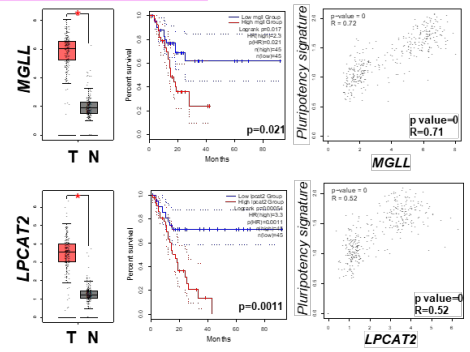
→ **AIM: To analyse the specific role of FA metabolism in the advanced and metastatic phenotype of PDAC.**

Material & Methods

- *Biological Material*: primary cultures from **PDAC Patient derived xenografts** (PDXs).
- *Bioinformatic analyses*: Expression data from human PDAC tissue and normal tissue analysed using the webserver GEPIA2 (TCGA and the GTEx project databases) or Oncomine™ (Badea, Buchholdz, Grutzmann, Iacobuzio-Donahue, Ishikawa, Logsdon, Pei, Segara databases). Correlation analyses were calculated by Pearson coefficient. Disease-free survival was analysed using the Cox Proportional Hazards model.
- *Inhibitors*: FA mitochondrial uptake (**Etomoxir** 200µM), FA oxidation (**Mildronate** 100µM, **Perhexiline** 1µM, **Ranolazine** 50µM) 48 or 72h.
- *Statistics*: ANOVA or Kruskall-Wallis tests, results were considered as significantly different if $p < 0.05$.

Results

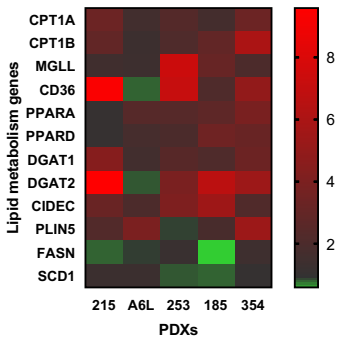
Bioinformatic analysis



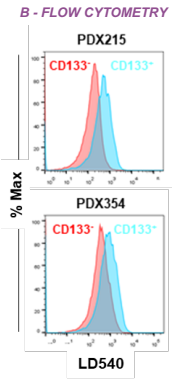
Lysophosphatidylcholine acyltransferase 2 (**LPCAT2**) and Monoglyceride lipase (**MGLL**), two main actors of Lipid Droplets (LD) dynamics, are **overexpressed in PDAC tumour tissues**, predicting a **lower survival rate** in patients. Interestingly, the expression of these genes **positively correlated with a stemness signature in PDAC**.

In CSC *vs* non-CSC

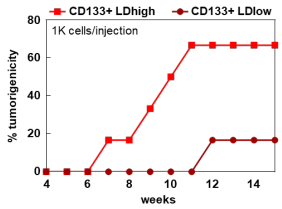
A - GENE EXPRESSION



B - FLOW CYTOMETRY



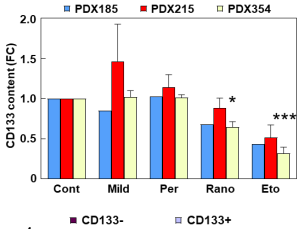
C - IN VIVO EXTREME LIMITING DILUTION ASSAY (ELDA)



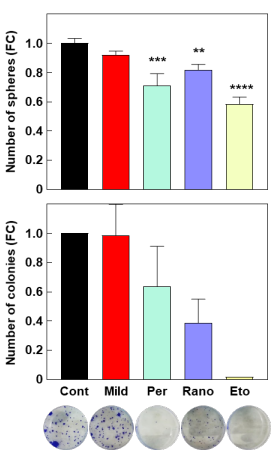
By using primary cultures of PDAC PDXs, we observed that **CSC-enriched cultures (spheres, CD133+) overexpressed lipid metabolism genes (A)** and showed **an increased LD content (B)**. Importantly, **CD133+ cells with high LD content were more tumorigenic *in vivo* (C).**

CSC Features in response to Lipid Metabolism Inhibitors

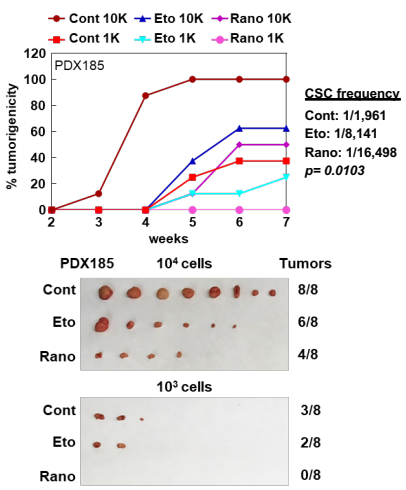
A - FLOW CYTOMETRY



B - SPHERE AND COLONY FORMATION ASSAYS

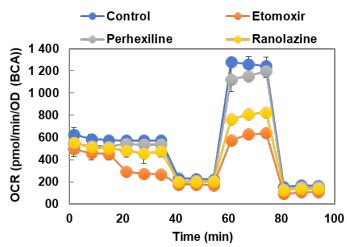


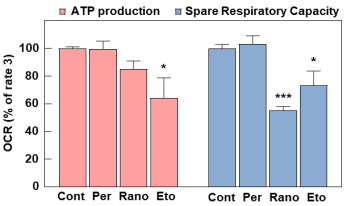
C - IN VIVO EXTREME LIMITING DILUTION ASSAY (ELDA)



The use of **inhibitors of FA mitochondrial uptake/oxidation** induced **cell death in CD133+ cells**, decreasing the percentage of this tumorigenic population **(A)**. Additionally, **these inhibitors strongly impaired CSC functionality measured as sphere or colony formation** *in vitro* **(B)**. *In vitro* pretreatment (48h) with this inhibitors **decreased the ability to form tumours *in vivo*** (subcutaneous injection of 10,000 or 1,000 cells), and demonstrated a **significant decrease of CSC frequency** following these treatments **(C).**

Mitochondrial stress test





Mechanistically, the acute blockade of FA metabolism strongly **decreased mitochondrial ATP-linked respiration and spare respiratory capacity**, likely leading to an **energy crisis** in the CSC population.

Conclusions

- Our results demonstrate a **strong reliance of PDAC CSC on lipid metabolism**, which could represent an interesting therapeutic avenue in order to eliminate this extremely tumourigenic population.

Funding: This work was supported by **Worldwide Cancer Research** and the Spanish Association Against Cancer (**AECC**)