ASSOCIATION OF LIPID DROPLETS ACCUMULATION WITH STEMNESS AND CHEMORESISTANCE IN PANCREATIC CANCER

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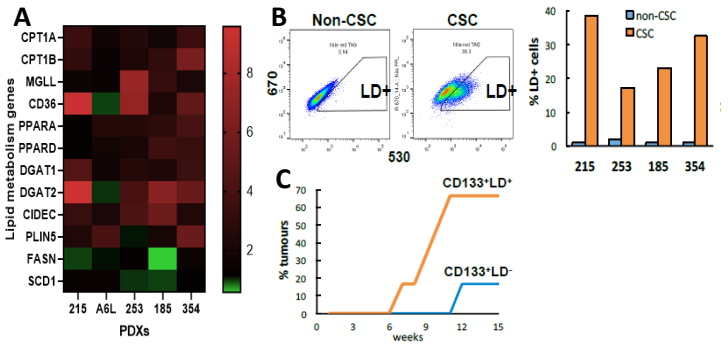
Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest tumours nowadays, with incidence predicted to increase over the next decade. Currently, surgery is the unique curative alternative available. PDAC intrinsic aggressiveness, chemoresistance and metastasis formation can be attributed to a subpopulation of cancer cells with stem-like properties, the so-called cancer stem cells (CSCs). These pancreatic CSCs are highly dependent on mitochondrial oxidative metabolism, which can be fuelled by several substrates, including fatty acids (FAs) through fatty acid oxidation (FAO). FAs can be also stored as triacylglycerides in intracellular organelles-called lipid droplets (LDs), serving as energy storage units for cancer cells.

MATERIALS AND METHODS: *Biological material:* Primary cultures of PDAC patient-derived xenografts (PDXs) and established PDAC cell lines (MiaPaca-2 and BxPC-3). *Oil Red staining. Proliferation assays. CSC functionality.* Sphere formation assay, *in vivo* extreme limiting dilution assay. *Quantitative PCR (qPCR). Flow Cytometry:* LD540 (lipid droplets), Nile Red (lipid droplets), Zombie Violet (cell viability) and anti-CD133. *Statistics:* ANOVA and Kruskal-Wallis, p-value < 0.05 was considered statistically significant.

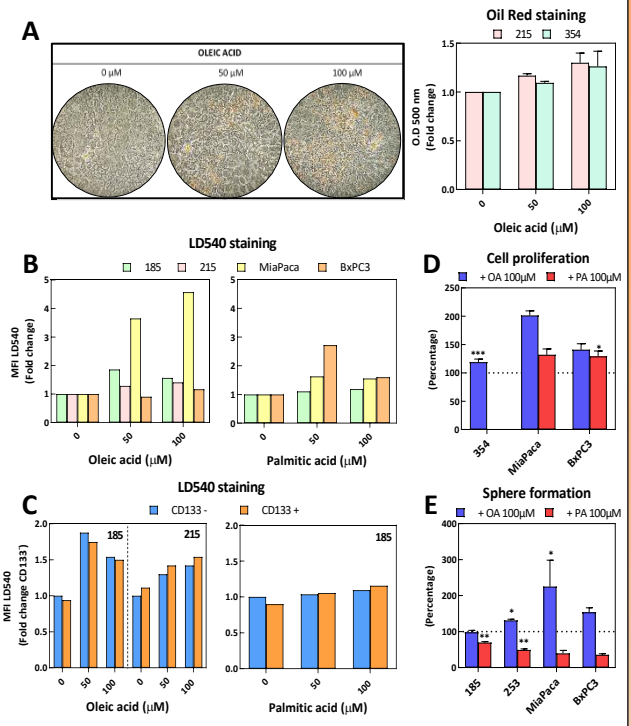
RESULTS: Our preliminary results show that PDAC CSCs, enriched as sphere cultures or identified with the stemness marker CD133, expressed higher levels of several lipid-related genes and accumulated lipids as intracellular deposits (LDs). Interestingly, CD133⁺ cells with higher LD accumulation showed enhanced tumorigenicity. Additionally, we observed an increase in lipid accumulation which was slightly more visible in CD133⁺ cells when culturing pancreatic cancer cells with exogenous palmitic and oleic FAs. Incubation with these exogenous FAs altered cell growth and self-renewal, measured as sphere formation capability. Incubation with the chemotherapeutic agent gemcitabine also induced lipid accumulation in pancreatic cancer cells, suggesting that lipid metabolism might be used as a marker of chemoresistance. Importantly, our results show that FAs supplementation also affected the response to chemotherapy on pancreatic cancer cells, further correlating lipid accumulation in LDs with chemoresistance.

Figure 1. Lipid metabolism and storage is increased in pancreatic CSCs



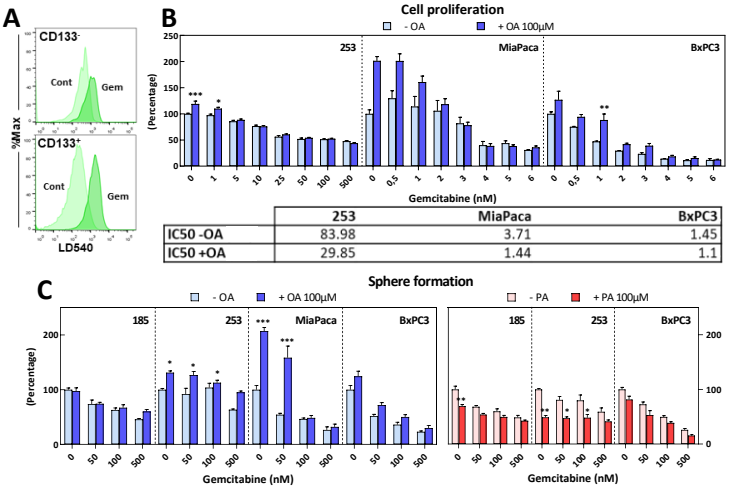
A. Heatmap of fold change in the expression of genes involved in lipid metabolism in CSC-enriched cultures vs. differentiated cells from different PDAC PDXs primary cultures. B. Left: Representative flow cytometry plots of non-CSC (adherent cells) and CSC-enriched cultures (sphere-derived cells) from PDX215 after Nile Red staining. Right: LD content in different PDXs C. Tumorigenicity of CD133+ cells sorted by LD content and subcutaneously implanted in nude mice (100 cells/injection). Data are represented as mean.

Figure 2. Effects of exogenous FAs on CSCs features



A. Oil Red staining of PDAC PDX primary cultures treated with exogenous OA at the indicated concentrations for 48h. Left: Representative image of PDX215 (x20 magnification) Right: Quantification. B, C. Quantification of LD content after LD540 staining by flow cytometry in cells treated with exogenous OA or PA for 48h; in the general population (B) and CD133- and CD133+ cells (C). D, E. Cell proliferation (D) and sphere formation (E) after treatment with exogenous OA or PA for 3 and 7 days, respectively. Data are represented as mean ± SEM. ANOVA with Bonferroni post-test or Kruskal-Wallis with Dunn's post-test. *p<0,5; **p<0,01; ***p<0,001.

Figure 3. Effects of exogenous FAs on chemotherapy



	253	MiaPaca	BxPC3
IC50 -OA	83.98	3.71	1.45
IC50 +OA	29.85	1.44	1.1

A. Representative flow cytometry plots of LD content measured by LD540 after treatment with gemcitabine for 48h, in CD133- (upper panel) and CD133+ (lower panel) cells. B. Upper panel: Cell proliferation after treatment with gemcitabine in combination with exogenous OA for 3 days. Lower panel: IC50 absolute values (nM). C. Sphere formation after treatment with gemcitabine in combination with exogenous OA or PA for 7 days. Data are represented as mean ± SEM. ANOVA with Bonferroni post-test or Kruskal-Wallis with Dunn's post-test. *p<0,5; **p<0,01; ***p<0,001.

CONCLUSION: Although further experimental work is needed, our results indicate an interconnection between lipid accumulation in LDs and stemness and chemoresistance in PDAC cells. If confirmed, lipid metabolism could represent an interesting target for the elimination of pancreatic CSCs.