Respiratory complex I deficiency triggers accumulation of lipid droplets and endoplasmic reticulum stress response

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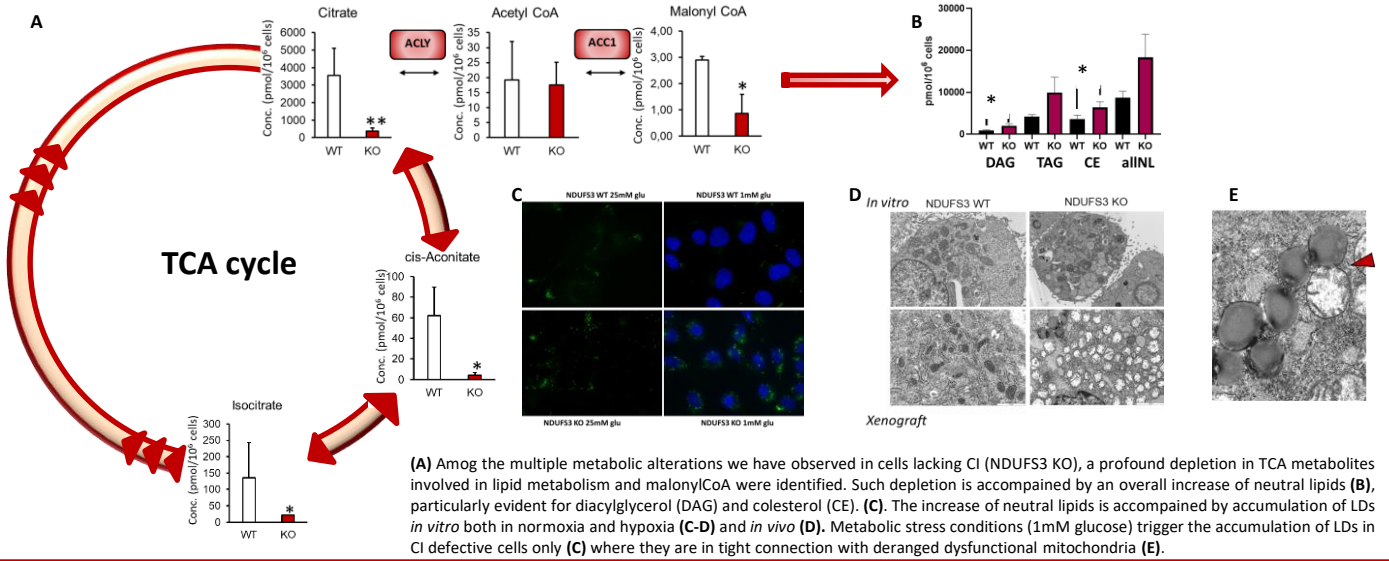
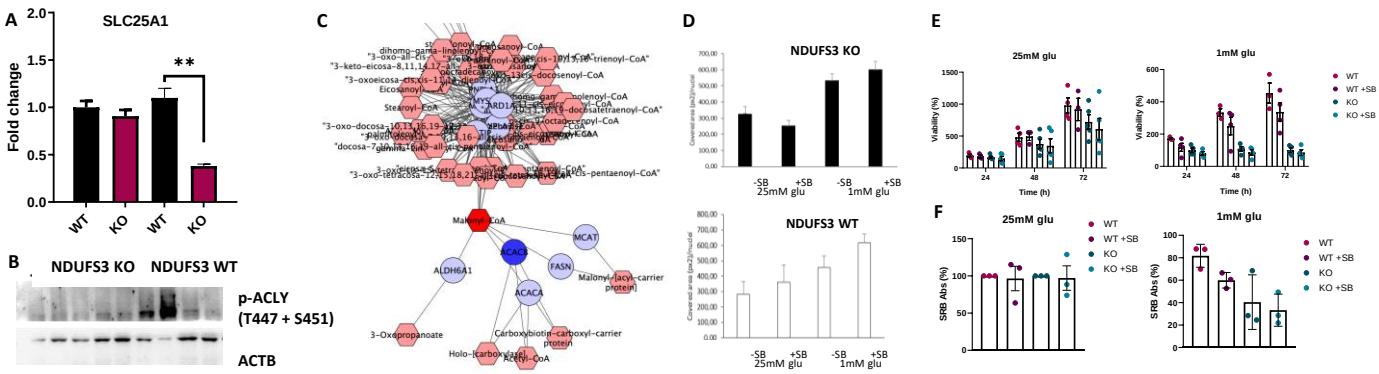
BACKGROUND and AIMS: Respiratory complex I (CI) is a major contributor to cancer progression by acting as an *oncojanus* according to the degree of its dysfunction [1]. Its severe impairment strongly delays tumor progression by causing a profound metabolic reprogramming which prevents hypoxic adaptation [2]. Such metabolic alteration involves the whole TCA cycle but also other metabolic pathways, including also amino acids metabolism. Two cell models (143B and HCT116) defective for respiratory CI have been investigated. They were fully characterized in terms of bioenergetics, hypoxic response and tumor progression [2]. Cells lacking CI showed a block in mitochondrial respiration accompanied by increased intramitochondrial oxygen levels, αKG accumulation, reduced stabilization of HIF1α and a delay in tumor growth *in vivo*. We pushed forward the biochemical investigation of these models performing a multi-omics analysis (targeted metabolomics, lipidomics and RNAseq). Moreover, lipid droplets abundance and features of lipid metabolism were investigated in preliminary experiments to dissect the role of lipids in CI defective models, also in correlation with markers of endoplasmic reticulum (ER) stress.

1. Lack of CI induces a profound alteration of lipid metabolism and accumulation of lipid droplets (LDs)



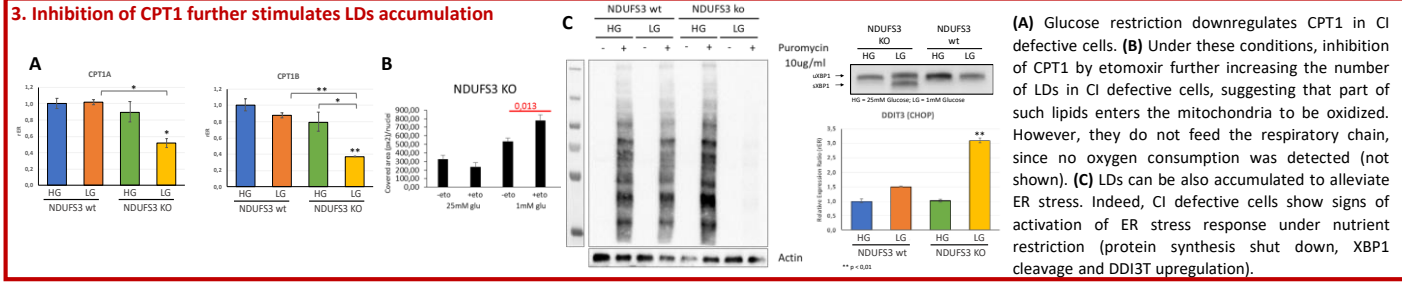
(A) Amog the multiple metabolic alterations we have observed in cells lacking CI (NDUFS3 KO), a profound depletion in TCA metabolites involved in lipid metabolism and malonylCoA were identified. Such depletion is accompanied by an overall increase of neutral lipids **(B)**, particularly evident for diacylglycerol (DAG) and colesterol (CE). **(C)**. The increase of neutral lipids is accompained by accumulation of LDs *in vitro* both in normoxia and hypoxia **(C-D)** and *in vivo* **(D).** Metabolic stress conditions (1mM glucose) trigger the accumulation of LDs in CI defective cells only **(C)** where they are in tight connection with deranged dysfunctional mitochondria **(E)**.

2. Lipid-related metabolites' depletion and LDs accumulation are not due to augmented de novo FAS



(A) The mitochondrial citrate transported SLC25A1 is downregulated in CI defective cells under metabolic stress (1mM glucose) and **(B)** ATP citrate lyase (ACLY) is phosphorylated and thus activated only in NDUFS3 WT xenografts, promoting the use of citrate for *de novo* FAS only in CI-competent cells. **(C)** Integrated network analysis of RNAseq and metabolomics data revealed that decreased levels of malonyl-CoA are associated with low levels of ACACB gene coding for acetyl-CoA carboxylase beta that converts acetyl-CoA to malonyl-CoA. Inhibition of ACLY with SB-204990 (SB) does not affect LDs accumulation **(D)**, cell viability **(E)** or colony formation **(F).**

3. Inhibition of CPT1 further stimulates LDs accumulation



(A) Glucose restriction downregulates CPT1 in CI defective cells. **(B)** Under these conditions, inhibition of CPT1 by etomoxir further increasing the number of LDs in CI defective cells, suggesting that part of such lipids enters the mitochondria to be oxidized. However, they do not feed the respiratory chain, since no oxygen consumption was detected (not shown). **(C)** LDs can be also accumulated to alleviate ER stress. Indeed, CI defective cells show signs of activation of ER stress response under nutrient restriction (protein synthesis shut down, XBP1 cleavage and DDI3T upregulation).

CONCLUSIONS: CI deficiency dramatically alters the metabolic status of cancer cells and triggers LDs accumulation. The molecular definition of LDs origin and role is still ongoing, pointing towards a compensatory accumulation likely due to the activation of ER stress response.

FINANCIAL SUPPORT

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