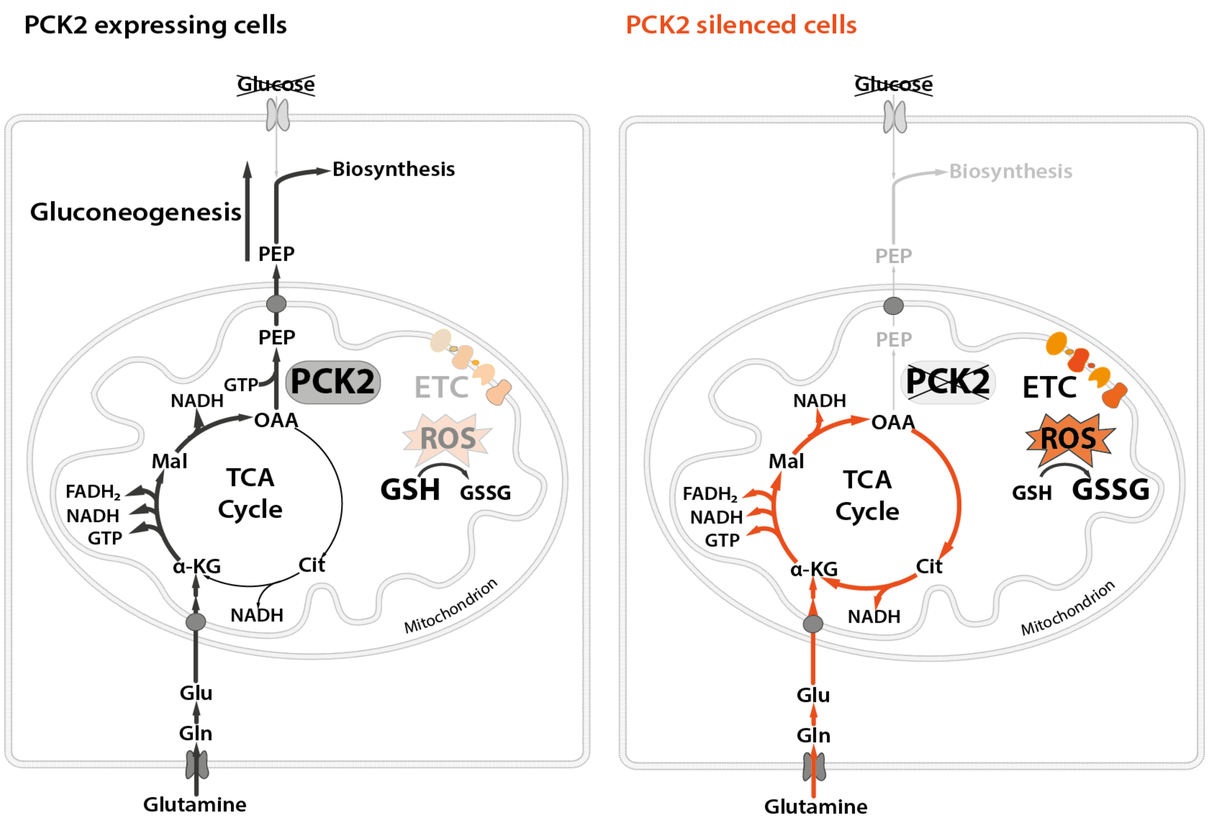
PCK2 balances TCA cycle flux and mitochondrial respiration to maintain the redox equilibrium in starved lung cancer cells

Gabriele Grasmann[1], Mélanie Planque[2], Corina T. Madreiter-Sokolowski[3,4], Wolfgang F. Graier[3], Tobias Madl[3,5], Anna Springer[3], Andelko Hrzenjak[1,6], Horst Olschewski[1,6], Sarah-Maria Fendt[2], Katharina Leithner[1]

[1] Division of Pulmonology, Department of Internal Medicine, Medical University of Graz, Graz, Austria
[2] Laboratory of Cellular Metabolism and Metabolic Regulation, VIB, KU Leuven Center for Cancer Biology, Leuven, Belgium
[3] Gottfried Schatz Research Center for Cell Signaling, Metabolism and Aging, Molecular Biology and Biochemistry, Medical University of Graz, Graz, Austria
[4] Energy Metabolism Laboratory, Institute of Translational Medicine, Department of Health Sciences and Technology, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland
[5] BioTechMed-Graz, Graz, Austria
[6] Ludwig Boltzmann Institute for Lung Vascular Research, Graz, Austria



PCK2 enables metabolic flexibility upon starvation

Introduction

Cancer cells undergo metabolic reprogramming in order to adapt to fluctuating nutrient availability. One up-regulated metabolic pathway is gluconeogenesis, the reversal of glycolysis. The key enzyme and bottle neck for gluconeogenesis is phosphoenolpyruvate carboxykinase (PEPCK) [1]. Our lab and others described that cancer cells metabolize small non-carbohydrate molecules into serine, purine nucleotides and the glycerol backbone of phospholipids via PCK2, the mitochondrial isoform of PEPCK under starvation conditions [2,3,4]. Silencing of PCK2 led to decreased cell viability *in vitro* and in *in vivo* xenograft models [3,4]. Here we show the importance of PCK2 beyond its necessity for the synthesis of glycolytic intermediates.

Summary

In summary, we show that PCK2 plays a cataplerotic role in lung cancer cells, inhibiting excessive respiration and the formation of ROS under starvation conditions. Accordingly, PCK2 inhibition significantly impaired colony formation by starved lung cancer cells. As a conclusion, PCK2 inhibition could potentially be utilized as a therapeutic approach to prevent metabolic adaptation and to enhance the formation of cell-damaging ROS in lung cancer cells.

RESULTS I – **PCK2 silencing increases TCA cycle intermediates and enhances respiration in starved lung cancer cells**

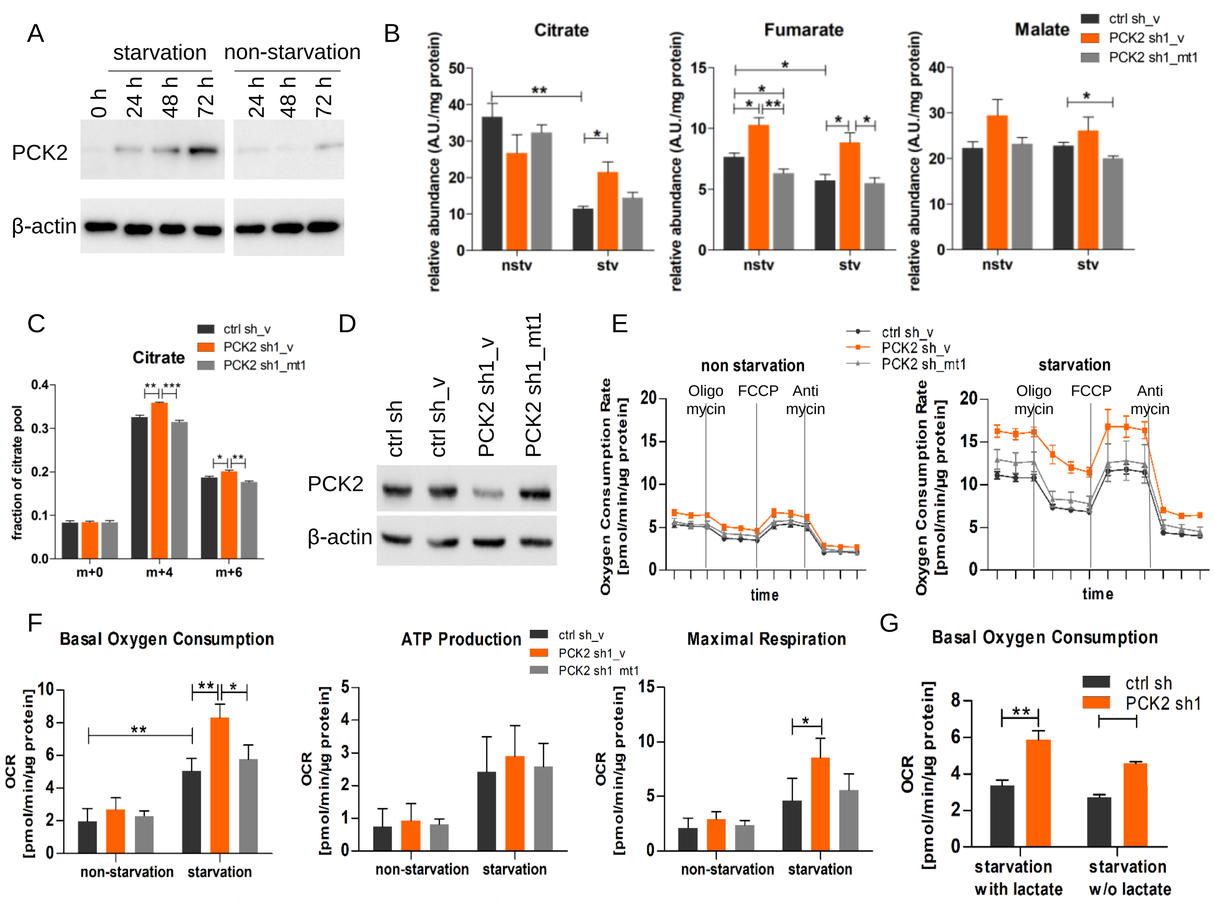


Figure 1. PCK2 expression, TCA cycle intermediates and mitochondrial respiration in H23 lung cancer cells. (A) PCK2 expression in H23 cells cultured under starvation (0.2 mM glucose, 0% dialyzed FCS (dFCS)) or non-starvation conditions (10 mM glucose, 10 % dFCS) for 0/24/48 and 72h. (B) H23 cells stably expressing Ctrl sh or PCK2 sh1 were transfected with the empty vector (ctrl sh_v and PCK2 sh1_v) or PCK2-sh1 resistant PCK2 (PCK2 sh1_mt1) for rescue and cultured for 24h before TCA cycle intermediates were measured with GC-MS and analyzed with Matlab. (C) H23 cells were transfected, treated and analyzed as described in (B), non-starvation and starvation media contained 2 mM of $^{13}C_5$ glutamine. (D,E,F) H23 cells were treated as described in (B) and (D) protein was collected and (E,F) oxygen consumption was measured with a Seahorse analyzer. (E) H23 cells stably expressing PCK2 shRNA (PCK2 sh1) or non-silencing shRNA (Ctrl sh) were treated with starvation medium containing either 0 mM (medium w/o lactate) or 10 mM lactate Results are shown as mean +/- SEM. *P<0.05 **P < 0.01 ***P < 0.01.

RESULTS II – **PCK2 silencing increases mitochondrial superoxide and decreases GSH/GSSG ratio. PCK2 silencing inhibits while antioxidants rescue colony formation under starvation**

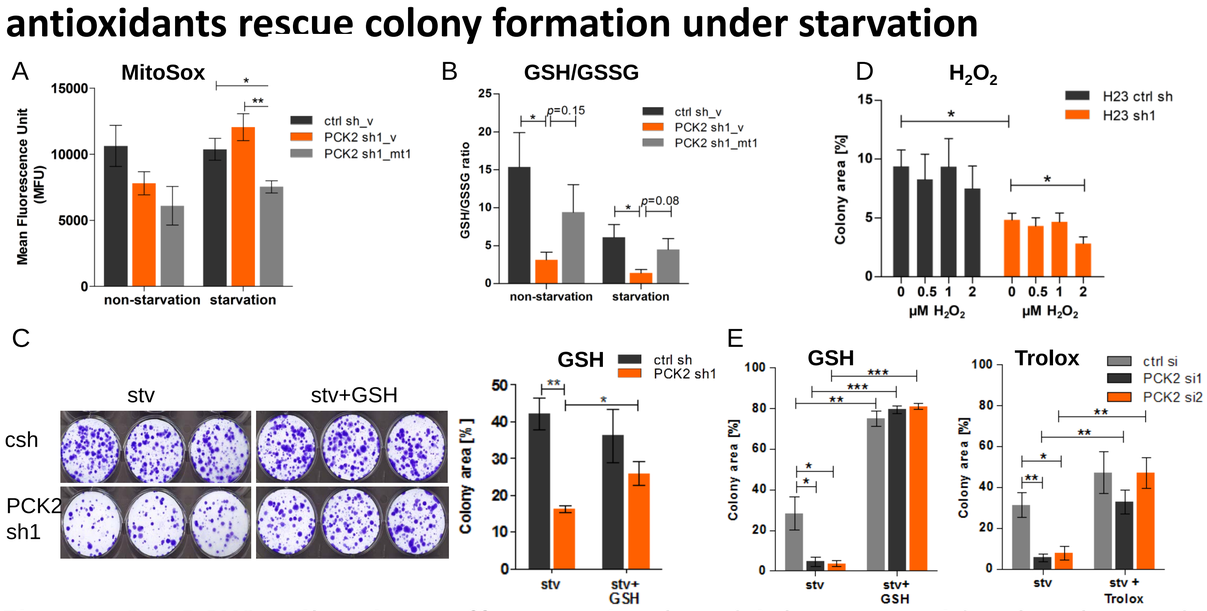


Figure 2. PCK2 silencing affects mitochondrial superoxide levels and GSH/GSSG ratio in H23 cells. Antioxidants rescue colony formation in H23 and A549 lung cancer cells. (A) H23 cells were treated as described in (Figure 1 A). Mitochondrial superoxide levels were detected with the MitoSox dye, (B) GSH/GSSG levels were measured using a commercially available kit. (C) H23 cells stably expressing PCK2 shRNA (PCK2 sh1) or non-silencing shRNA (Ctrl sh) were cultured under starvation conditions, if indicated (C) 2 mM GSH or (D) different amounts of H_2O_2 were added. After 72 h of treatment, media was changed to normal growth media to allow colony formation to proceed. Colony area was measured with ImageJ. (E) A549 cells transfected with either a PCK2 silencing siRNA (PCK2 si1/PCK2 si2) or a non-silencing siRNA (ctrl si) and cultured under starvation conditions, if indicated 100 µM Trolox or 2 mM GSH were added, colony formation was performed as described in (C). Results are mean +/- SEM. *P<0.05 **P < 0.01.

RESULTS III – **Additon of the TCA cycle intermediate Dimethyl L-malate phenocopies PCK2 silencing**

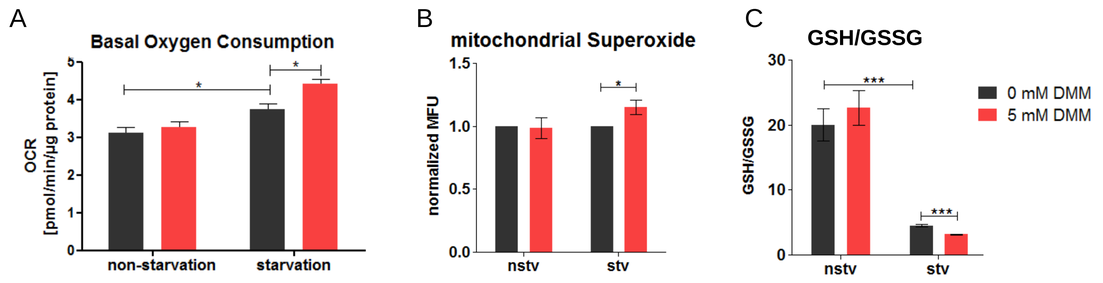


Figure 3. Dimethyl L-malate (DMM) phenocopies effects of PCK2 silencing. (A,B,C) H23 cells were cultured under non-starvation or starvation conditions, if indicated 5 mM DMM were added. (A) oxygen consumption was measured with the Seahorse analyzer, (B) mitochondrial Superoxide was measured with the MitoSox dye and flow cytometry and (C) GSH/GSSG ratio was determined with a commercially available kit. Results are mean +/- SEM. *P<0.05, **P<0.01, ***P<0.001

Funding:

ÖAW ÖSTERREICHISCHE AKADEMIE DER WISSENSCHAFTEN

FWF Der Wissenschaftsfonds.

References:

(1) Grasmann G et al. BBA 2019; 1872 (1): 24-36
(2) Leithner K et al. Oncogene 2015; 34(8):1044-50
(3) Vincent EE et al. Mol Cell 2015; 60(2):195-20
(4) Leithner K et al. PNAS 2018; 115(24):6225-30