Mitochondrial serine metabolism mediates 5-fluorouracil resistance in colorectal cancer by fueling nucleotide biosynthesis and supporting DNA damage response

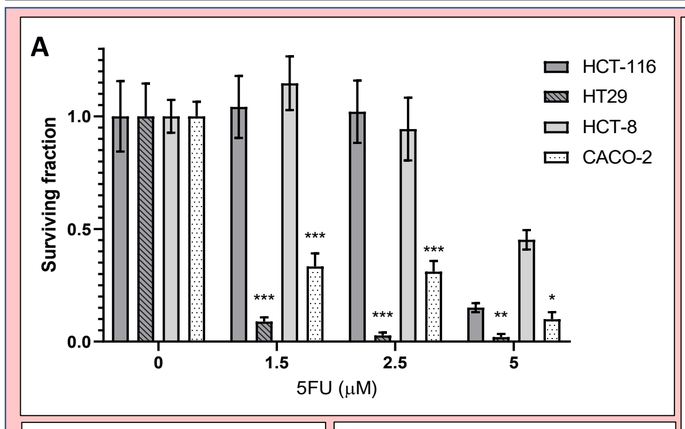
Erica Pranzini[1], E. Pardella[1], A. Leo[1], I. Nesi[1], L. Mucillo[2], T. Zhang[3], M. Parri[1], T. Lottini[1], V. Colantuoni[2], P. Cirri[1], P. Chiarugi[1], O.D.K. Maddocks[3], P. Paoli[1], M.L. Taddei[4]

[1]Department of Experimental and Clinical Biomedical Sciences, University of Florence, Viale Morgagni 50, 50134 Florence, Italy.; [2]Department of Sciences and Technologies, University of Sannio, 82100 Benevento, Italy; [3]Institute of Cancer Sciences, Wolfson Wohl Cancer Research Centre, University of Glasgow, Switchback Road, Glasgow, G61 1QH, UK.; [4]Department of Experimental and Clinical Medicine, University of Florence, Viale Morgagni 50, 50134 Florence, Italy.

Despite constant progress towards the development of effective anticancer strategies, drug resistance remains a major obstacle to treat cancers as the great plasticity of cancer cells often leads to the emergence of resistant clones. Several studies recently underlined the implication of metabolic reprogramming in supporting drug resistance, pointing out that cancer cells can rewire their metabolism in response to the treatment resulting in a resistant phenotype. Recognizing the specific metabolic adaptations supporting the survival to a given drug is of primary importance for developing effective approaches to prevent and target therapy resistance.

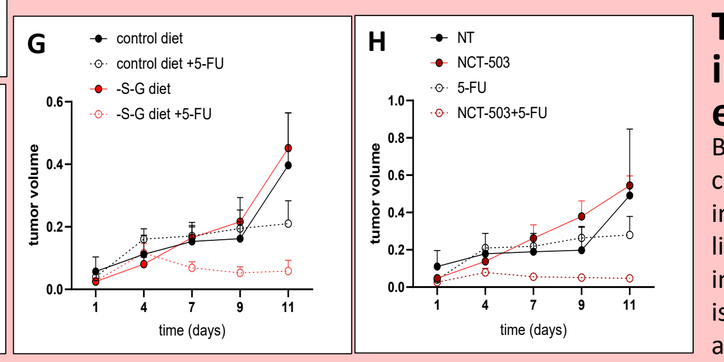
5-fluorouracil (5-FU) is a primary chemotherapeutic agent for the management of multiple solid malignancies, including advanced and metastatic colorectal cancer (CRC)

Here we outline a pivotal role of serine metabolism in mediating 5-FU resistance in CRC by supporting nucleotide biosynthesis. In particular, we identify specific compartmentalization of serine-derived carbons metabolism inside the mitochondria of 5-FU resistant CRC cells mediating purine biosynthesis and supporting DNA-damage repair. These results indicate that interfering with serine utilization could be a valid strategy to potentiate 5-FU efficacy in CRC treatment and re-synthesize resistant cancer cells to drug toxicity.



Serine derived from both endogenous synthesis and exogenous up-take drives 5-FU response in CRC

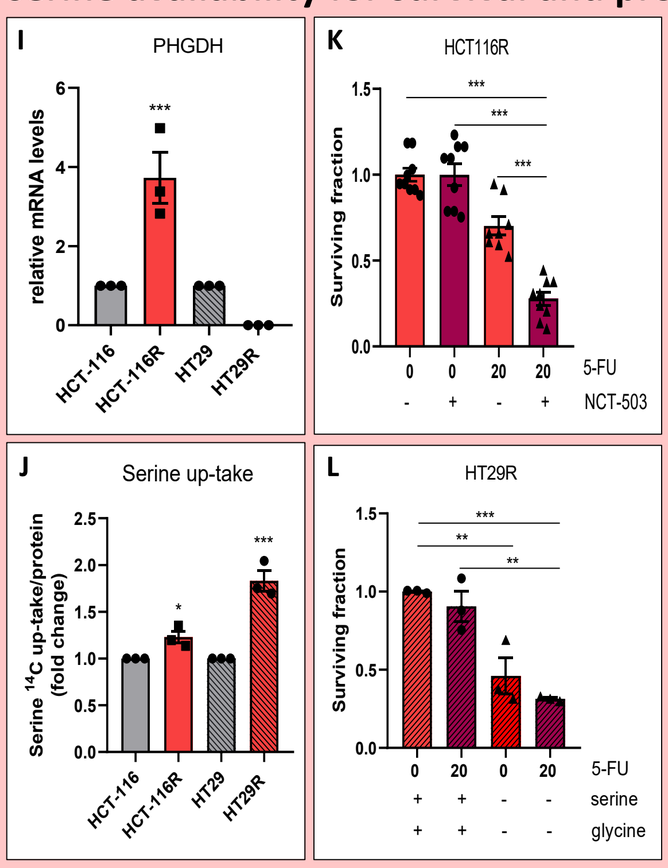
By analyzing four CRC cell lines characterized by a differential sensibility to 5-FU (A), we found substantial differences in the expression of PHGDH, the first rate-limiting enzyme of the serine synthesis pathway (B). By culturing CRC cell lines with [1-2^{14}C]-serine, we measured cellular incorporation of exogenous serine, and we found that PHGDH-low cells display higher levels of serine uptake than PHGDH-high cells (C). To determine the contribution of serine to 5-FU adaptive response, we investigated the effect of serine and glycine withdrawal on cell proliferation under 5-FU exposure. Cells were let to proliferate in standard medium (+ser +gly) or medium lacking serine and glycine (-ser-gly) in the presence or not of non-lethal doses of 5-FU (D). The absence of serine and glycine in the culture medium affects all the analyzed cell lines' proliferation and combining serine/glycine starvation with 5-FU treatment further reduces their growth rate (D). Treating the PHGDH-high cells with NCT-503, a specific PHGDH inhibitor, increases the susceptibility of these poor-responder cells to the treatment with 5-FU (E-F).



Targeting serine availability increases 5-FU anticancer effects *in vivo*

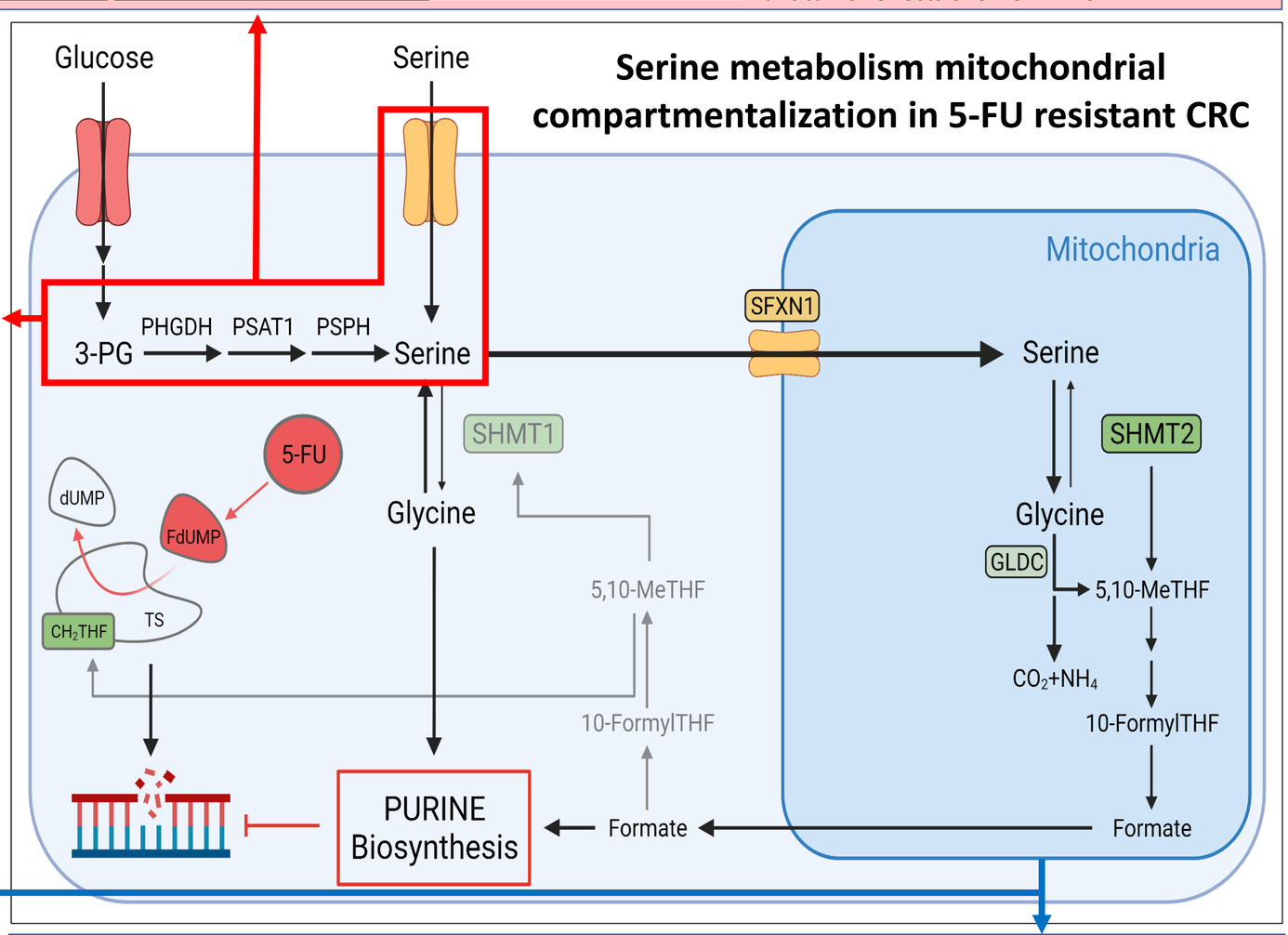
By using a syngeneic mouse model of CRC (CT26 cells injected in Bab/c mice), we found that interfering with serine metabolism by both limiting circulating serine amount (by dietary intervention)(G) or inhibiting PHGDH activity (H) is an effective strategy to potentiate the antitumor effects of 5-FU *in vivo*.

Selected 5-FU resistant CRC cells are strictly dependent on serine availability for survival and proliferation



To investigate serine dependency of CRC cells displaying acquired drug resistance, we developed *in vitro* models of 5-FU resistance by treating HCT-116 and HT29 CRC cell lines with increasing concentrations of 5-FU for six months resulting in the selection of two resistant clones perfectly tolerant to the treatment with up to 20 μM of 5-FU ("HCT-116R" and "HT29R"). 5-FU acquired resistance confers a strong dependency on serine availability, which is achieved by resistant cells either by enhancing endogenous serine synthesis (HCT-116R)(I) or increasing exogenous serine uptake (HT29R)(J), depending on the genetic background and the basal PHGDH expression.

Targeting serine metabolism is sufficient to rescue 5-FU sensibility in resistant clones. NCT-503 treatment restores 5-FU sensitivity in HCT-116R cells (K), and depleting HT29R cells of exogenous serine and glycine potentiates the efficacy of 5-FU in affecting resistant cells survival (L).



5-FU resistance is supported by mitochondrial compartmentalization of one-carbon metabolism

By real-time PCR analysis, we found a substantial decrease in SHMT1 (M) and an increase in SHMT2 (N) in resistant cells derived from both cell lines. Confirming a shift toward the mitochondrial branch of one-carbon metabolism, we found enhanced mRNA levels of the primary mitochondrial serine transporter SFXN1 in resistant cells derived from both cell lines (O). Remarkably, real-time PCR analysis also revealed an increase in glycine decarboxylase (GLDC) (P) which catalyzes the complete degradation of glycine in CO2, NH4+ and 5,10-MeTHF inside the mitochondria.

By investigating mitochondrial OXPHOS reliance, we found enhanced oxygen consumption rate (OCR) in resistant than parental cells. Concomitant deprivation of exogenous serine and glycine decreases OCR in resistant cells, while it doesn't affect OCR in parental cells. Notably, the sole serine supplementation is sufficient to rescue OCR in resistant cells (data not shown)

Serine supports purine biosynthesis and DNA-damage response in 5-FU resistant CRC cells

Following serine-derived carbons, we found that serine mainly supports purine biosynthesis in resistant cells as demonstrated by increased production of m+2 IMP following [U-^{13}C]-serine incubation (Q). The quantification of histone H2AX phosphorylation proves that resistant clones from both cell lines display enhanced ability to prevent DNA damage accumulation following 5-FU exposure, while corresponding parental clones do not (R). Targeting serine metabolism by treating with NCT-503 (S) or limiting exogenous serine (T) enhances 5-FU-induced DNA damage accumulation in resistant cells. Modulating SHMT1/2 balance affects 5-FU response: SHMT1 deletion increases colony-forming ability after 5-FU treatment in HCT116 cells (U) while silencing SHMT2 in HCT116R cells restores 5-FU susceptibility (V).

A shift toward mitochondrial serine metabolism promotes 5-FU resistance in CRC by supporting purine nucleotide biosynthesis and allowing resistant cells to prevent drug-induced DNA damages. Mitochondrial compartmentalization of one-carbon metabolism supports 5-FU resistance by (1) decreasing cytosolic CH_2-THF, thereby leading to reduced formation of the inhibitory ternary complex on TS by 5-FU derivatives, and (2) supporting the synthesis of purine nucleotides to prevent DNA damage accumulation under drug exposure.