**Cancer Metabolism: Addicted to serine**

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**Phosphoglycerate dehydrogenase (PHGDH) is an enzyme of serine biosynthesis overexpressed in various types of cancer. A new series of PHGDH inhibitors selectively block proliferation of PHGDH-dependent cancer cells and revealed an unexpected role of serine biosynthesis in coordinating one-carbon metabolism.**

Cancer cells are biosynthetic factories and gear multiple metabolic pathways towards cell growth and proliferation. Besides glucose and glutamine, serine is the third most consumed metabolite by cancer cells[1], and it is used as building block for proteins and as carbon donor for nucleotide biosynthesis. Serine is a non-essential amino acids and can be synthesised *de novo* from glucose (Figure 1, left panel). Studies in the late 1980s demonstrated that *de novo* synthesis of serine is increased in cancer cells[2], suggesting that this pathway might be relevant for cancer cell growth. It was not until the landmark discovery that phosphoglycerate dehydrogenase (*PHGDH*), the first step of *de novo* serine synthesis, is amplified in breast cancer[3] and melanoma[4], that this pathway came to the limelight. Importantly, silencing PHGDH in PHGDH-dependent cancers significantly affects their growth, making this enzyme an excellent target for cancer therapy. Recent work from Mullarky *et al* reported the discovery of a novel non-competitive inhibitor of PHGDH[6]. However, although this compound was selective against PHGDH-dependent melanoma and breast cancer cell lines, it was unstable in mouse plasma, limiting its use *in vivo*. In this issue of Nature Chemical Biology, Pacold *et al.* discovered small molecule inhibitors of PHGDH that exhibit potent antitumor activity both *in vitro* an *in vivo*[7].

*PHGDH* is now considered a *bona fide* oncogene and besides its amplification, its transcriptional activation by the antioxidant master regulator Nuclear factor (erythroid-derived 2)-like 2 (NRF2) was recently observed in lung cancer[5]. Inhibition of PHGDH decreases the growth and survival of PHGDH-dependent cancer cell lines even when extracellular serine is plentiful [3-5] This observation raised the possibility that PHGDH and glucose-derived serine metabolism have additional roles for cancer cells, besides providing building blocks for protein and nucleotide biosynthesis. In support of this hypothesis, it was shown that PHGDH harbours promiscuous enzymatic activity and catalyses the reduction of aKG to the oncometabolite 2-hydroxyglutarate[10], which might explain the oncogenic function of PHGDH. Furthermore, it was observed that serine biosynthesis, via PSAT-dependent aKG production, contributes to up to 50% of the anaplerotic flux from glutamine[3], emerging as a major determinant of mitochondrial function. Of note, both these functions are exquisite by-products of *de novo* serine synthesis and are independent of extracellular supply of serine. Yet, none of these studies investigated the fate of extracellular serine after silencing of PHGDH.

To tackle this outstanding question, Pacold *et al.* set out to identify novel PHGDH inhibitors. Similar to Mullarky et al(REF) the authors developed an *in vitro* enzymatic assay to measure PHGDH activity by coupling the production of NADH generated by the conversion of 3-phosphoglycerate (3PG) to phosphohydroxy-pyruvate (pPyr) (Figure 1, light brown insert) to the diaphorase-mediated reduction of resazurin. They identified two potent piperazine-1-carbothioamide PHGDH inhibitors, NCT-502 and NCT-503, and an "inactive compound" with a similar structure but with no activity against PHGDH. When tested on cell lines and xenograft tumours, PHGDH inhibitors exhibited a very potent anticancer activity against PHGDH-dependent cell lines but had not effects on PHGDH-independent counterparts, underlining their efficacy and on-target effect. Besides a very specific inhibition of serine biosynthesis, PHGDH inhibitors did not perturb the abundance of any other amino acids, with the exception of aspartate, found to be depleted upon PHGDH inhibition. This unexpected result revealed a mild mitochondrio-toxic activity of the two PHGDH inhibitors, shared also by the inactive compound.

To examine the role of PHGDH for serine biosynthesis, Pacold *et al.* performed a comprehensive book-keeping of carbons from $^{13}C$-labelled serine and glucose using liquid chromatography mass-spectrometry. They found that PHGDH inhibition not only affects serine biosynthesis from glucose but also reduces the incorporation of carbons from both extracellular and intracellular serine into nucleotides (Figure 1, right panel). Importantly, nucleotide depletion was mechanistically linked to cell cycle arrest observed upon PHGDH inhibition. In light of these results, Pacold *et al.* hypothesised that PHGDH inhibitors cause a mishandling of one-carbon units generated from serine. Indeed, additional functional experiments showed that when PHGDH is inhibited, the cytosolic enzyme Serine Hydroxymethyltransferase 1 (SHMT1), instead of extracting one-carbon units from serine to support nucleotide biosynthesis, regenerates serine from glycine instead, wasting one-carbon units (Figure 1, right panel). Consistently, deletion of SHMT1 using CRISPR-based genome editing was sufficient to redirect one-carbon units from serine to nucleotides biosynthesis and restored proliferation defects upon PHGDH inhibitors.

Overall, the work presented by Pacold *et al.* uncovered a new facet of serine metabolism in PHGDH-dependent cancer cells. However, few unanswered questions still remain. For instance, the authors did not investigate the mechanism of action of the novel PHGDH inhibitors. Their data indicate a non-competitive mode of inhibition with respect to PHDH substrates, 3-PG and $NAD^+$. This mechanism of action, similar to that of CBR-5884, the PHGDH inhibitor developed by Mullarky et al (REF), supports the presence of an allosteric pocket of PHGDH. It would be interesting to assess whether these compounds affect the oligomeric state of PHGDH, as shown for CBR-5884. From a biological point of view, the presented results demonstrate that in PHGDH-dependent cancer cells glucose-derived serine biosynthesis coordinates one-carbon utilisation and, unexpectedly, SHMT1 directionality. It is tempting to speculate that metabolites generated within serine biosynthesis exert a regulatory function on SHMT1. However, the details of this feedback mechanism are still unknown. Finally, these results suggest that cancer cells can discriminate intra- and extracellular serine. It is possible that this type of selective usage of substrates is achieved by a tight compartmentalization of the underpinning metabolic pathways. Hopefully, the compounds developed by Pacold *et al.* will shed some light on this fascinating conundrum of cell metabolism.

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**Figure Legend.**

**Figure 1. Schematic representation of serine metabolism and effects of PHGDH inhibitor NCT-503.** In untreated PHGDH-dependent cells, glucose is diverted towards serine biosynthesis to provide one-carbon units for nucleotide synthesis and aKG for anaplerosis. PHGDH inhibitor NCT-503 selectively blocks glucose-derived serine synthesis but at the same time triggers SHMT1-dependent one-carbon units wasting to synthesize serine from glycine. This futile cycle depletes the cell of nucleotides and leads to cell cycle arrest. The light brown insert in the left panel indicates the three enzymes used for the *in vitro* PHGDH-inhibitors screening. Pyr=pyruvate; 3PG=3-phosphoglycerate; pPyr=phosphohydroxy-pyruvate; pSer=phosphoserine; PSAT= phosphoserine aminotransferase; PSPH= phosphoserine phosphatase; akG=alpha-ketoglutarate; THF=tetrahydrofolate; meTHF= 5,10-methylene THF; fTHF=formyl THF; SHMT1/2= serine hydroxymetyl transferase 1/2; TCA cycle=tricarboxylic acid cycle