

A novel small-molecule inhibitor of 3-phosphoglycerate dehydrogenase

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ABSTRACT

Serine metabolism is likely to play a critical role in cancer cell growth. A recent study reports the identification of a novel small-molecule inhibitor of serine synthesis that targets 3-phosphoglycerate dehydrogenase (PHGDH), the first enzyme of the serine synthesis pathway, and selectively abrogates the proliferation of PHGDH overexpressing breast cancer cells.

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Although the importance of serine for tumorigenesis has been recognized historically, recent developments have led to a remarkable resurgence of interest in this *a priori* anodyne amino acid.¹ Serine is a non-essential amino acid given that it can be synthesized *de novo* in mammalian cells. However, serine is more aptly characterized as a conditionally essential amino acid in that the cellular demand for serine can exceed the capacity for synthesis and thus render the cell dependent on extracellular serine import.² Serine synthesis proceeds via the diversion of glycolytic flux into the phosphoserine pathway (Fig. 1). This pathway consists of three sequential enzymatic reactions, with the first committed step being catalyzed by 3-phosphoglycerate dehydrogenase (PHGDH).³ Interestingly, there is significant heterogeneity in the activity of the pathway across cancer cell lines.⁴ High pathway activity stems from PHGDH overexpression and induces a dependence on serine synthesis such that proliferation of PHGDH overexpressing cells is inhibited by PHGDH knockdown. Conversely, cancer cell lines that express little or no PHGDH are auxotrophic for serine and insensitive to inhibition of the pathway.⁴⁻⁷ Given that ample serine is present in the culture media and readily available for import, it is surprising that PHGDH overexpressing cells are sensitive to inhibition of the pathway.

Multiple mechanisms for PHGDH overexpression have been reported including focal amplification of *PHGDH*, which is particularly present in melanomas and breast cancers.^{5,6} Interestingly, in non-small cell lung cancer, oncogenic activation of the transcription factor NRF2 (nuclear factor erythroid-2-related factor 2) signaling axis, via mutation of *NRF2* or its negative regulator *KEAP1* (Kelch-Like ECH-Associated Protein 1), has recently been found to activate serine synthesis.⁴ Somatic mutations that disrupt NRF2 signaling are found in a variety of

cancers, raising the tantalizing possibility that *de novo* serine synthesis might be an attractive target across a broad range of cancers.⁴

The exact mechanism underlying why certain cancer cells are addicted to serine synthesis remains unclear. However, given that serine is critical for a plethora of anabolic processes, one would suspect that defects in serine synthesis would impair cell biomass production. Serine is directly incorporated into the head-groups of certain lipids and is an abundant constituent of proteins (Fig. 1). Moreover, serine hydroxymethyltransferases (SHMTs) catabolize serine to glycine and a methylene one-carbon unit used to charge the intracellular folate pool.³ Both glycine and folate one-carbon units are consumed to produce nucleotides. In addition, by providing adenosine and folate one-carbon units for homocysteine remethylation serine supports production of S-adenosylmethionine (SAM), the major methyl group donor for methylation reactions including those that define epigenetic states.⁸ Interestingly, mitochondrial folate metabolism has been shown to be an important source of NADPH, the major intracellular reducing currency that powers cellular antioxidant defenses and reductive biosynthesis.^{9,10} Given that serine is the major donor of methyl units for the folate cycle this raises the possibility that serine synthesis may be critical for maintaining cellular NADPH levels.⁴

The unique sensitivity of PHGDH overexpressing cancer cell lines to inhibition of the pathway and the identification of focal amplifications of the gene encoding PHGDH in human cancer specimens suggested that PHGDH may be a clinically attractive target in cancers.^{5,6} Therefore, a library of 800,000 drug-like small molecules was screened using a coupled *in vitro* enzymatic assay for PHGDH.⁷ Following hit confirmation and counter screening against the coupling enzyme, a total of 408 PHGDH inhibitors were identified. To select for compounds that were

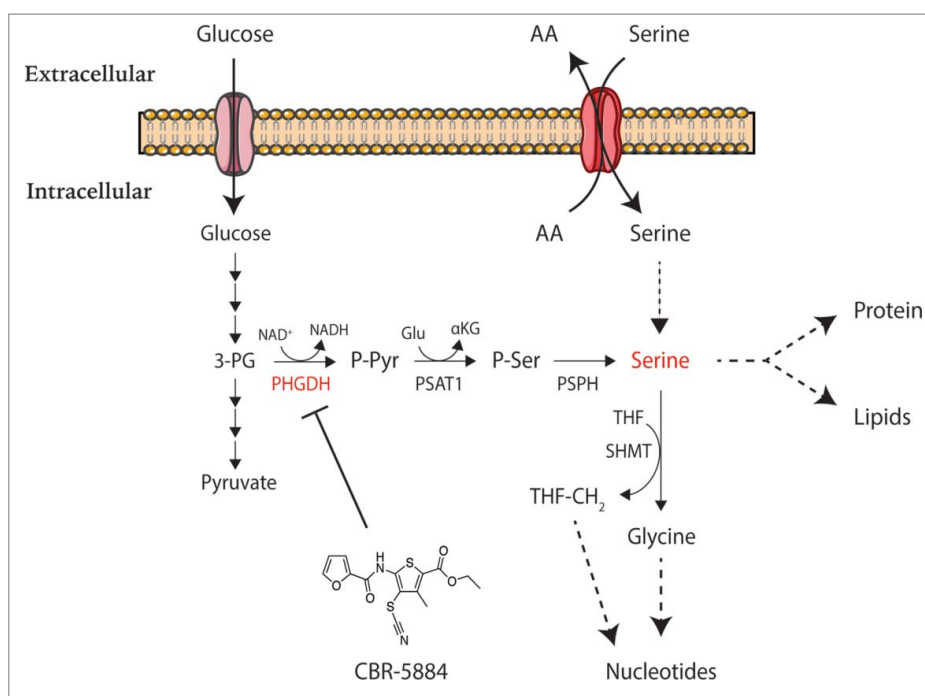


Figure 1. The serine synthesis pathway and downstream anabolic reactions of serine. Serine can be imported from the extracellular space by amino acid exchangers that trade extracellular serine for an intracellular amino acid (AA). Alternatively, serine can be synthesized *de novo* from glucose via the phosphoserine pathway in three reactions: first, PHGDH oxidizes 3-phosphoglycerate (3-PG) using an NAD^+ cofactor to give phosphohydroxy pyruvate (P-Pyr); second, phosphoserine aminotransferase (PSAT1) transaminates P-Pyr to phosphoserine (P-Ser) using glutamate (Glu) as the nitrogen donor; finally, phosphoserine phosphatase (PSPH) hydrolyzes P-Ser to serine. Serine is directly incorporated into proteins and the head groups of certain lipids. Serine hydroxymethyltransferases (SHMTs) catabolize serine to glycine and methylene tetrahydrofolate (THF-CH_2) thus charging the intracellular folate pool. Both serine-derived folate one-carbon units and glycine contribute to nucleotide synthesis. The newly identified compound CBR-5884 specifically inhibits PHGDH to abrogate serine synthesis.

specific inhibitors of PHGDH, the activity of the 408 hits was profiled against a panel of NAD(P)^+ -dependent dehydrogenases. Based on this triaging strategy, the most potent PHGDH-specific inhibitors progressed to cell-based assays. To establish pharmacological proof-of-concept, the ability of the inhibitors to impair cellular *de novo* serine synthesis in an acute setting was determined using $^{13}\text{C}_6$ -glucose tracing into serine. Acute treatment was preferred over longer-term treatment to guard against false positives that decrease serine synthesis by a mechanism other than PHGDH inhibition. For example, decreased serine synthesis could arise as an artifact of cell death if a compound was generally cytotoxic. Of the seven inhibitors tested in cells, compound CBR-5884 was the most effective inhibitor of serine synthesis. Importantly, dose response experiments showed that CBR-5884 selectively blocked serine synthesis without affecting other glycolytic intermediates, indicating that reduced serine synthesis is a direct consequence of CBR-5884 treatment.

Profiling the activity of CBR-5884 on a panel of breast cancer cell lines grown in serine-containing media showed that CBR-5884 was selectively toxic to cells that overexpress PHGDH without affecting cell lines that express little or no PHGDH. Removing serine from the media to increase the reliance of cells on serine synthesis exacerbated the antiproliferative effect of CBR-5884 on PHGDH overexpressing cells. Importantly, PHGDH knockdown phenocopied the effects of CBR-5884 treatment in terms of both the selective toxicity toward PHGDH overexpressing cells and the increased efficacy under serine depleted conditions. Further biochemical characterization revealed that CBR-5884 was a noncompetitive inhibitor with respect to both

PHGDH substrates (NAD^+ and 3-PG) and showed a time-dependent onset of inhibition. It was also demonstrated that drug treatment destabilized the tetrameric form of PHGDH in favor of the dimeric species. These results are interesting because they suggest that PHGDH might be regulated by transitions between the dimeric and tetrameric states *in situ*; as exemplified by pyruvate kinase M2, modulation of metabolic enzyme activity by affecting quaternary structure is well documented.¹⁰

Overall, the identification of a novel small-molecule inhibitor of PHGDH that is selectively toxic to cancer cells that overexpress PHGDH provides a proof-of-concept that targeting serine synthesis in cancer is a viable and attractive strategy in oncology. In addition, CBR-5884 will serve as a valuable tool to study serine metabolism in both normal and cancerous cells.

Disclosure of potential conflicts of interest

L.C.C. owns equity in, receives compensation from, and serves on the Board of Directors and Scientific Advisory Board of Agios Pharmaceuticals. Agios Pharmaceuticals is identifying metabolic pathways of cancer 135 cells and developing drugs to inhibit such enzymes in order to disrupt tumor cell growth and survival.

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