Targeting one-carbon metabolism requires mTOR inhibition: a new therapeutic approach in osteosarcoma

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ABSTRACT

The rate-limiting enzyme of serine biosynthesis, 3-phosphoglycerate dehydrogenase (PHGDH), contributes to rapid growth and proliferation when it is overexpressed in cancer. We recently described the metabolic adaptations that occur upon PHGDH inhibition in osteosarcoma. PHGDH inhibition causes metabolite accumulation that activates the mechanistic target of rapamycin (mTOR) signaling, sensitizing osteosarcoma to non-rapamol mTOR inhibition.

PHGDH has become an exciting target for the development of novel cancer therapeutics as it is the rate-limiting enzyme in de novo serine biosynthesis and a key contributor to the oxidized nicotinamide adenine dinucleotide (NAD+) salvage pathway. PHGDH is highly expressed in several cancers, including breast cancer, melanoma, Ewing’s sarcoma, and osteosarcoma. Small molecule inhibitors of PHGDH, including NCT-503 and PKUMDL-WQ-2101, were utilized in these cancers and could decrease cellular proliferation; however, these inhibitors may have limited clinical viability due to a lack of on-target cell death induction as a monotherapy. Building off previous work in the field to understand the utilization of glucose and serine in cells treated with PHGDH inhibitors, Rathore et al. explored the metabolic adaptations that occur in osteosarcoma cells because of PHGDH inhibition. NCT-503 treatment affected several metabolites within one-carbon metabolism and adjacent pathways. The authors found that when de novo serine biosynthesis was blocked, glucose-derived 3-phosphoglycerate was no longer incorporated into serine, but rather stayed within glycolysis to accumulate ultimately as lactate. The lack of glucose flux through serine biosynthesis results in decreased lipid, protein, and nucleic acid synthesis, thereby decreasing cellular capacity for proliferation. Furthermore, mitochondrial energy production decreased upon PHGDH inhibition, resulting in the accumulation of non-glucose-derived acetyl-coenzyme A (acetyl-coA) and unsaturated fatty acids.

The lack of mitochondrial oxidative phosphorylation, accumulation of fatty acids, and decreased purine and pyrimidine metabolism suggested that nutrient sensing pathways that were activated by the lack of de novo serine biosynthesis were implicated in the survival mechanism for cells treated with PHGDH inhibitors. The authors found that activating transcription factor 4 (ATF4), the transcriptional regulator of serine metabolism, is one of the newest hallmarks of cancer due to the efficient alterations in cellular energetics and biomass production that are required for cancer initiation and progression. The catabolic and anabolic processes of tumor metabolism are readily adaptable to keep up with the high energy and biomass demands of rapidly proliferating tumors. As such, tumor metabolism is an evolving target for the development of novel oncology therapies.

The serine/folate pathways are a set of interconnected pathways leading to one-carbon metabolism that play a critical role in tumor progression. The glycolytic intermediate 3-phosphoglycerate is converted into the amino acid serine by the serine biosynthetic pathway, after which a single carbon from serine is transferred into the folate cycle. The folate cycle uses the single carbon unit to recycle diet-derived folate and tetrahydrofolate, producing pyrimidines and purines for DNA and RNA synthesis. The folate cycle is then linked to the methionine cycle by the enzyme methionine synthase, which continues utilizing the single carbon unit to produce S-adenosylmethionine (SAM), the source of methyl units for epigenetic regulation. The amino acids serine, glycine, and methionine contribute to protein synthesis and amino acid homeostasis. The rate-limiting serine biosynthetic enzyme, 3-phosphoglycerate dehydrogenase (PHGDH), also produces reduced nicotinamide adenine dinucleotide (NADH) in its enzymatic reaction, and the folate and methionine cycles contribute to the homeostasis of oxidized/reduced nicotinamide adenine dinucleotide phosphate (NADP+/NADPH). The upregulation of one-carbon metabolism by cancer cells generates nucleic acids and proteins and maintains redox and epigenetic homeostasis to support rapid tumor growth and proliferation. In a recent publication in Cell Reports, Rathore et al. explored the consequences of inhibiting these pathways in osteosarcoma.
biosynthesis, was upregulated with NCT-503 treatment, suggesting that osteosarcoma cells attempted to compensate for small-molecule inhibition of PHGDH by further increasing expression of serine biosynthetic enzymes. The mechanistic target of rapamycin complex 1 (mTORC1) pathway is responsible for integrating nucleotide and other nutrient sensing cues to drive ATF4 expression, suggesting that PHGDH inhibition could be activating mTORC1 signaling in osteosarcoma to compensate for the loss of serine synthesis in osteosarcoma.

The authors further described the accumulation of SAM upon PHGDH inhibition, further evidence of the lack of recycling in the folate and methionine cycles. The accumulation of SAM has also been linked to the activation of mTORC1 through the SAM-sensing protein complex SAMTOR. When intracellular SAM levels are elevated, the SAMTOR protein complex is released from the lysosome, allowing for the inhibition of the GATOR1 protein complex, and the subsequent activation of mTORC1.

To test mTORC1 activation as a pro-survival metabolic mechanism, the authors combined PHGDH inhibition by NCT-503 with rapamycin, the canonical mTORC1 inhibitor, and surprisingly found no combinatorial or synergistic effect on cell death. Notably, rapamycin and other rapalogs are the first generation of mTORC1 inhibitors. The authors then explored second generation mTORC1 inhibitors, known as non-rapalogs, which inhibit the kinase activity of mTORC1 by targeting the ATP-competitive binding domain, and identified perhexiline as a candidate. Perhexiline, a small molecule inhibitor traditionally utilized as a carnitine palmitoyltransferase 1 (CPT1) inhibitor at high concentrations, is a mTORC1 inhibitor at lower concentrations. The authors found that NCT-503 combined with perhexiline was able to cause synergistic cell death in osteosarcoma cell lines and was able to decrease cell line-derived xenograft outgrowth significantly in vivo.

Critically, perhexiline has already been approved outside of the US for the treatment of chronic refractory angina, while PHGDH inhibition awaits phase one testing. This publication demonstrates the pre-clinical justification for the combination of small molecule inhibition of PHGDH with perhexiline or other non-rapalog mTORC1 inhibitors for the treatment of PHGDH-positive osteosarcoma. A novel phase one approach may be required to develop this combination, as neither agent may have a strong effect individually. This dual metabolic therapy has the potential to be clinically effective in not just osteosarcoma, but also other PHGDH-overexpressing cancers.

**References**

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