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Therapeutic Startegies for Cancer Sub-Types Overexpressing Cps1

Nefertiti Muhammad and Jiyeon Kim*

Department of Biochemistry and Molecular Genetics, University of Illinois, USA

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Corresponding author:
Jiyeon Kim,
Department of Biochemistry and Molecular Genetics, University of Illinois, Chicago, 900 S. Ashland Ave. Chicago IL 60607, Tel: 1-312-996-9545l; Email: jiyeonk@uic.edu

ABSTRACT

Carbamoyl Phosphate Synthetase 1 (CPS1) catalyzes the first and rate-limiting step of the urea cycle. The urea cycle is normally used for ammonia detoxification, exclusively in liver; however, specific cancer subtypes are dependent on CPS1 for pyrimidine biosynthesis and not for ammonia detoxification. Novel small molecule inhibitors of CPS1 were recently identified and shown to have in vitro efficacy against the urea cycle and pyrimidine biosynthesis. We discuss how these small molecule inhibitors can be used to target cancer subtypes overexpressing CPS1 and the challenges employing them in vivo.

INTRODUCTION

Metabolism is altered in cancer cells to meet the demands of rapid proliferation: ATP synthesis, biomass production, and redox balance. Reprogramming central carbon metabolism is a common alteration in malignant cells. This discovery was built on the observations of Otto Warburg, who observed high glycolytic activity irrespective of oxygen in malignant cells [1]. ATP production was the accepted rationale for increased glycolytic activity in these cells, but current research has shown it serves more of a biosynthetic function. Instead of entering the TCA cycle, glucose carbons can be funneled into adjacent pathways related to amino acid and DNA biosynthesis. Alanine can be generated from pyruvate through a transaminase reaction with other amino acids. Through multiple reactions, 3-phosphoglycerate (3PG) can be converted into serine and further into glycine, critical amino acids for purine biosynthesis. The high glycolytic activity of cancer cells and the subsequent shunt of glucose carbons into amino acid biosynthesis has pushed cancer metabolism's focus beyond central carbon metabolism.

The dependency on glucose metabolism for amino acid synthesis is a vulnerability that can be targeted for therapeutics. Phosphoglycerate dehydrogenase (PHGDH) catalyzes the rate-limiting step of the serine/glycine biosynthesis pathway and is upregulated in a subset of lung adenocarcinoma, breast, and colorectal cancer [2-4]. Increased expression of PHGDH is associated with nucleotide biosynthesis and proliferation, as serine carbons are funneled into nucleotide biosynthesis pathways. PHGDH inhibitors (e.g., CBR-5884, NCT-502, NCT-503) were previously identified and have proven effective in vitro [5-7]. Malignant cells are also dependent on glutamine catabolism for proliferation. Glutamine carbons are funneled into the TCA cycle, while the nitrogens can be used for amino acid and nucleotide biosynthesis. To be utilized, glutamine is converted to glutamate and alpha-ketoglutarate through

glutaminolysis. Unsurprisingly a key enzyme in glutaminolysis, glutaminase (GLS), has been a popular target for therapy. GLS inhibitors, bis-2-[5-phenylacetamido-1, 2, 4-thiadiazol-2-yl] ethyl sulfide (BPTES), 968, and CB-839, have been mostly effective in vitro [8,9].

Increased glutamine catabolism in malignant cells fuels proliferation but produces toxic ammonia. The urea cycle mediates the removal of ammonia from the blood that comes from amino acid catabolism. The cycle is functional almost exclusively in the liver, where ammonia is biochemically turned into urea for secretion. In some cancer subtypes, this cycle is upregulated and essential for survival. Carbamoyl phosphate synthetase 1 (CPS1) is a great example. It catalyzes the first and rate-limiting step of the urea cycle in the mitochondria. CPS1 combines bicarbonate and ammonia to produce Carbamoyl Phosphate (CP) for non-toxic urea synthesis. The role of CPS1 in cancer is quite different from its original role in the liver. While some cancer cells use CPS1 to support proliferation by increasing cycle activity to prevent the accumulation of toxic ammonia within tumors, other cancers depend on CPS1-mediates CP for the pyrimidine biosynthesis, repurposing the urea cycle to support cell proliferation [10-12]. According to The Cancer Genome Atlas (TCGA), CPS1 is highly overexpressed in multiple cancer subtypes including lung, colon, prostate, bladder, esophageal, and endometrial cancers [13]. In non-small cell lung cancer (NSCLC), loss of function mutation of tumor suppressor STK11, which encodes protein kinase LKB1, induces CPS1 levels [10-12]. Two highly aggressive subtypes of NSCLC, one with concurrent mutations of oncogenic KRAS and LKB1 [10] and the other with oncogenic EGFR and LKB1 [11], show high levels of CPS1. Among other pathways, the two subtypes become dependent on CPS1 for pyrimidine biosynthesis. These recent findings provide a rationale for developing a compound targeting CPS1, which can meet the goal of precision medicine.

Yao et al. identified small molecule inhibitors targeting a previously unknown allosteric binding pocket of CPS1 [13]. CP is synthesized in two steps, on separate domains of CPS1. The first step occurs on the ATP A domain where bicarbonate is phosphorylated to CP and aminated into carbamate. This step consumes ATP and ammonia. ATP A activity relies on binding of the cofactor N-acetyl glutamate (NAG) which affects the conformation of its domain. In the second step, carbamate is transferred to the ATP B domain, where it is phosphorylated to CP, consuming a second ATP. The CPS1 inhibitor, H3B-120, competes with the substrate ATP and induces a conformational change in the ATP A domain (Figure 1). Together, this inhibits ATP hydrolysis and phosphorylation of bicarbonate. H3B-120 was shown to bind a previously unknown allosteric binding site distinctive from another CP producing enzyme, CAD (Carbamoyl-phosphate synthetase 2, Aspartate transcarbamylase, and Dihydroorotase). H3B-120 is specific to CPS1 and has virtually no effect on CPS2 due to differences in hydrophobicity between the two binding pockets. Yao et al. tested the efficacy of H3B-120 in vitro and in vivo assays. In hepatocyte cells, H3B-120 was able to impair the urea cycle by decreasing urea secretion into the medium. It was also able to impair pyrimidine biosynthesis, seen from decreased incorporation of stable isotope-labeled ammonia into pyrimidines.

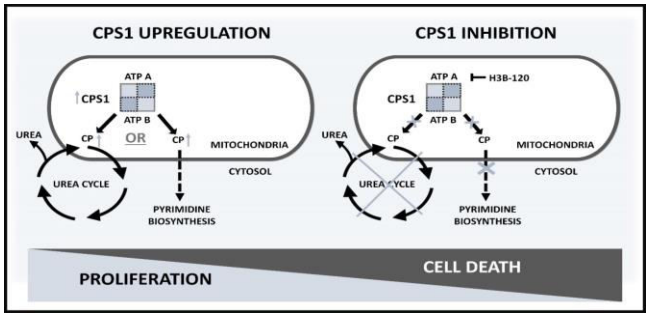

Figure 1: CPS1 Inhibition Decreases Proliferation in Cancer Subtypes Overexpressing CPS1 Cancer subtypes overexpressing CPS1 rely on the urea cycle to safely dispose of toxic ammonia or produce carbamoyl phosphate to feed pyrimidine biosynthesis for proliferation. CPS1 inhibitor, H3B-120, inhibits the ATP A domain of CPS1, impairing the urea cycle and pyrimidine biosynthesis. CPS1, carbamoyl phosphate synthetase 1; CP, carbamoyl phosphate; ATP A, CPS1 domain synthesizing the carbamate intermediate; ATP B, CPS1 domain synthesizing the carbamoyl phosphate product.

The identification of these small molecule inhibitors may present a therapeutic strategy for precision medicine. Cancer subtypes overexpressing CPS1 show selective sensitivity to CPS1 suppression (impaired growth, DNA damage, and cell cycle arrest), which makes them a favorable target for therapy.

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H3B-120 specifically targets one of the ATP-binding domains of CPS1 and not CPS2 of the CAD enzyme, a critical enzyme in conventional pyrimidine pathway for all types of cells. This bypasses a common problem of off-target effects and toxicity during clinical trials. Thus, the small molecule inhibitors targeting CPS1 are very promising for designing a therapeutic response to these cancer subtypes.

A significant challenge of targeting metabolism with small molecules is the lack of in vivo efficacy. PHGDH inhibitor CBR-5884, has yet to enter clinical trials for this reason and requires chemical optimization to enhance its potency [5]. The unsatisfactory in vivo stability, potency, and solubility of the GLS inhibitor, BPTES, also makes it a poor prospect for clinical therapy [9,14]. In the case of CPS1 inhibitor H3B-120, the half-life needs to be increased to be applicable in clinical trials. H3B-120 can only be stable for 40 minutes in cellular assays, which is sub-optimal for assays that require longer incubation periods. If H3B-120 cannot be stabilized for long periods, creating another inhibitor to target the NAG binding domain could be an attractive alternative.

Since CPS1 activity is crucial for the detoxification of ammonia, liver toxicity becomes another barrier that must be addressed. A specific drug delivery system is needed to avoid ammonia toxicity. Antibodies chemically linked to biologically active drugs called antibody drug conjugates (ADC), present a new way to target malignant cells. Two ADCs are in clinical trials and an ADC targeting glypican-3 (GPC3) in hepatocellular cancer is currently being explored in vitro and in vivo as a potential therapy [15].

Malignant cells are metabolically flexible and often respond to drug treatment by upregulating complementary metabolic pathways. This response was seen when targeting GLS, where pancreatic ductal adenocarcinoma was able to overcome CB-839 and BPTES inhibition by upregulating alternative pathways (i.e., micropinocytosis) [16]. When these pathways are upregulated to overpower the effects of the compound, combination therapy is often administered. These alternative pathways can be targeted to sensitize the cells to the initial therapy. Multiple-omics data of the cancer subtypes can be employed to identify metabolic barriers to H3B-120 inhibition. With that information, a proper drug combination therapy can be strategized to sensitize the cells to H3B-120. Cisplatin is often combined with the drug therapy in cancer cells targeted for nucleotide biosynthesis; to increase the cellular demand for nucleotides by inducing DNA damage. Identifying effective drug combinations with H3B-120 in vitro and in vivo would be the next step towards clinical applications.

Targeting metabolism with small molecule inhibitors is a favorable alternative to cytotoxic chemotherapy. In the case of H3B-120, drug delivery, liver toxicity, and in vivo efficacy are formidable barriers to clinical application. Despite these challenges, H3B-120's specificity to CPS1 and targeting of favorable cancer subtypes make it a potential therapeutic treatment. All in all, the identification of H3B-120 is very promising and a crucial step towards treating CPS1-dependent cancers.

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