AUTHOR'S VIEW

The folate cycle is a new metabolic weakness of cancer

Derek Lee^a and Carmen Chak-Lui Wong^{a,b}

^aDepartment of Pathology, The University of Hong Kong, Hong Kong; ^bState Key Laboratory for Liver Research, The University of Hong Kong, Hong Kong

ABSTRACT

Cancer cells harness nutrients to support its rapid proliferation through metabolic reprogramming. Our report identified folate cycle as a metabolic target in hepatocellular carcinoma. Folate cycle stagnation via inhibition of its mitochondrial folate enzyme methylenetetrahydrofolate dehydrogenase 1-like (MTHFD1L) greatly induced oxidative stress improving HCC cells' response to sorafenib.

Abbreviations: ETC, electron transport chain; FDA, Food and Drug Administration; HIF-1, Hypoxia-inducible factor 1; HCC, hepatocellular carcinoma; MTHFD1L, methylenetetrahydrofolate dehydrogenase 1-like; MTX, methotrexate; NADPH, Nicotinamide adenine dinucleotide phosphate; NDUFA4L2, NADH dehydrogenase (ubiquinone) 1 α sub-complex 4-like 2; NRF2, nuclear factor (erythroid-derived 2)-like 2; PPP, pentose phosphate pathway; redox, reduction-oxidation; ROS, reactive oxygen species; TKT, transketolase

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Hepatocellular carcinoma (HCC) is the most common form of liver cancer which remains largely incurable. Challenges for effective HCC treatment are mainly due to late presentations of symptoms as well as the lack of effective cure. Administration of sorafenib, the only targeted therapy with Food and Administration (FDA) approval, has shown limited hope, providing patients with additional survival benefits of only 3 months.¹ The liver is responsible for many important metabolic processes. Reprogramming of these metabolic processes drives the development of HCC. Targeting these metabolic processes may identify novel therapeutic method for HCC patients with better outcome.

Cancer cells, compared with normal cells, undergo proliferation rapidly. This represents much greater nutrient demands. To fulfill these demands, cancer cells, which are hugely addicted to glucose, will undergo aerobic glycolysis. This process famously known today as the “Warburg Effect” occurs through metabolic reprogramming and it allows maximal production of molecules beneficial for cancer cell survival.² Driven by this ground-breaking observation from Otto Warburg, cancer metabolism has gained greater attention with metabolic reprogramming now being considered a new hallmark of cancer.³

The roles of aerobic glycolysis in cancer cells go beyond providing energy to sustain rapid cell growth as antioxidant production also takes place. In comparison with normal cells, another distinct attribute of cancer cells is their constant generation of reactive oxygen species (ROS). Properties within the tumor microenvironment such as hypoxia induces ROS and eventually oxidative stress.⁴ Our understanding of ROS in cancer cells is rather controversial over the last few decades. ROS

was originally believed to benefit cancer cell growth. Relatively moderate (physiologically tolerable) amounts of ROS act as signaling molecules to promote tumor formation. This belief was eventually changed when it was observed that elevation in ROS levels induce irreparable damages to cancer cells, causing cell death.⁵ Intracellular oxidative stress results from the imbalance between ROS production and removal. Higher capacity for antioxidant production allows cancer cells to adapt and survive oxidative stress.⁶ Metabolic reprogramming coordinates greater productions of antioxidants in cancer cells and the relationship between metabolic alterations of HCC and reduction-oxidation (redox) homeostasis should be addressed. In our report, we effectively demonstrated that by exploiting metabolic vulnerabilities in HCC cells, we may utilize accumulated ROS to fight cancer cells itself.⁷

In our recent publication in *The Journal of Clinical Investigation*, we identified folate to be another nutrient, besides glucose and glutamine, required for HCC cell growth (Fig. 1A).⁷ We observed a decline in HCC proliferation upon withdrawal of folate from the culture media. Many metabolites produced from the folate cycle are vital for HCC growth. Interestingly, we learned that the folate cycle is a major producer for nicotinamide adenine dinucleotide phosphate (NADPH), of all metabolic processes.⁸ NADPH is an important antioxidant in humans, indicating that the folate cycle plays an important role in maintaining redox homeostasis in HCC.⁷

During the selection of a specific target within the folate cycle, we discovered methylenetetrahydrofolate dehydrogenase 1-like (MTHFD1L) to be most upregulated in human HCC samples.⁷ Additionally, MTHFD1L was also found to be

Figure 1. Schematic overview of the folate cycle and our study rationale using MTHFD1L-inhibition. (A) The folate cycle comprises the cytoplasmic and mitochondrial compartments. Production of major metabolites from its compartments essential for cell growth include nicotinamide adenine dinucleotide phosphate (NADPH), an antioxidant vital for counteracting oxidative stress; and thymidylate (dTTP), methionine, purines important for DNA synthesis and methylation. Folate derivative shuttles a single carbon unit through the tetrahydrofolate (THF) backbone in both compartments of the folate cycle. The folate cycle is interconnected through the shuffling of formate, serine, and glycine. Inhibition of the folate cycle member(s) may therefore result in halting the cycle. (B) Upregulation of MTHFD1L in human hepatocellular carcinoma (HCC) is driven by the transcription activation from nuclear factor (erythroid-derived 2)-like 2 (NRF2). Disruption of the folate cycle genetically via MTHFD1L knockdown and/or knockout, and/or pharmacologically using a folate inhibitor methotrexate (MTX) reduces antioxidant production, induces intracellular oxidative stress, and ultimately inhibits HCC growth. The folate cycle represents an attractive therapeutic target which induces vulnerability in HCC cells, rendering greater responsiveness toward sorafenib for better treatment outcomes in patients. Abbreviations: 10-CHO-THF: 10-formyl-THF; ALDH1L1/2: 10-formyl-THF dehydrogenase; CH⁺-THF: 5,10-methenyl-THF; CH₂-THF: 5,10-methylene-THF; CH₃-THF: 5-methyl-THF; MTHFD1: methylene-THF dehydrogenase 1; MTHFD1L: MTHFD1-Like; MTHFD2: methylene-THF dehydrogenase 2; MTHFD2L: MTHFD2-Like; MTHFR: methylenetetrahydrofolate reductase; NAD⁺/NADH: nicotinamide adenine dinucleotide; SHMT1/2: serine hydroxymethyltransferase 1/2; ROS: reactive oxygen species; TYMS: thymidylate synthase.

upregulated in other solid cancers and this further indicates its importance in cancer.⁷ Upregulation of MTHFD1L was found to be through the transcriptional activation of nuclear factor (erythroid-derived 2)-like 2 (NRF2)⁷ (Fig. 1B), a master activator of antioxidant genes under oxidative stress.⁹ These findings demonstrate that metabolic reprogramming in cancer cells produce antioxidant for neutralizing its high capacity of ROS.

Our study then followed by demonstrating the knockdown and/or knockout of MTHFD1L in HCC cells consistently decreased NADPH production which allowed ROS accumulation.⁷ Induction of intracellular oxidative stress eventually causes adverse effects like cell cycle delay and cell death (i.e. apoptosis) (Fig. 1B).⁷ We previously identified the electron

transport chain (ETC) complex I subunit NADH dehydrogenase (ubiquinone) 1 α subcomplex 4-like 2 (NDUFA4L2) plays an important role in preventing ROS accumulation. The hypoxia inducible factor-1 (HIF-1) transcriptionally activated NDUFA4L2 to reduce mitochondrial activities, thus the electron flux through the ETC, to reduce ROS.¹⁰ Together with our current study, we showed that cancer cells could reduce ROS generation and increase antioxidant production to maintain low oxidative stress levels for survival.

Through the inhibition of MTHFD1L in our study, we discovered the disruption of the folate cycle would inhibit HCC growth. The most clinically relevant findings of our study were the synergism between folate cycle inhibition and sorafenib administration in HCC treatment.⁷ MTHFD1L-knockdown HCC cells displayed greater response toward sorafenib, evident from greater cell apoptosis and tumor growth suppression (Fig. 1B).⁷ Pharmacological inhibition of the folate cycle was used in our study to inhibit HCC cell growth, demonstrating its applicability as a potential strategy for HCC treatment in clinic.⁷ The combined administration of methotrexate (MTX)—an anti-folate agent—with sorafenib achieved similar results (Fig. 1B).⁷ Previously, our group identified the inhibition of the pentose phosphate pathway (PPP) by targeting the crucial enzyme transketolase (TKT) decreased NADPH production. The resulting ROS accumulation led to adverse effects to HCC cells and increased sensitivity toward sorafenib.⁹ Both our studies demonstrated the high dependence of HCC cells on NADPH and highlight the importance for cells to undergo metabolic reprogramming upon cancer development. Further identification of metabolic processes as therapeutic targets will be interesting as targeting combinations of metabolic mechanisms may elicit an effective therapeutic strategy for cancer.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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References

- Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; 359(4):378-90; PMID:18650514; <https://doi.org/10.1056/NEJMoa0708857>
- Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; 144(5):646-74; PMID:21376230; <https://doi.org/10.1016/j.cell.2011.02.013>
- Ward PS, Thompson CB. Metabolic reprogramming: a cancer hallmark even warburg did not anticipate. *Cancer Cell* 2012; 21(3):297-308; PMID:22439925; <https://doi.org/10.1016/j.ccr.2012.02.014>
- Szatrowski TP, Nathan CF. Production of large amounts of hydrogen peroxide by human tumor cells. *Cancer Res* 1991; 51(3):794-8; PMID:1846317
- Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. *Nat Rev Cancer* 2011; 11(2):85-95; PMID:21258394; <https://doi.org/10.1038/nrc2981>

6. Gorrini C, Harris IS, Mak TW. Modulation of oxidative stress as an anticancer strategy. *Nat Rev Drug Discov* 2013; 12(12):931-47; PMID:24287781; <https://doi.org/10.1038/nrd4002>
7. Lee D, Xu IM, Chiu DK, Lai RK, Tse AP, Lan Li L, Law CT, Tsang FH, Wei LL, Chan CY, et al. Folate cycle enzyme MTHFD1L confers metabolic advantages in hepatocellular carcinoma. *J Clin Invest* 2017; 127(5):1856-72; PMID:28394261; <https://doi.org/10.1172/JCI90253>
8. Fan J, Ye J, Kamphorst JJ, Shlomi T, Thompson CB, Rabinowitz JD. Quantitative flux analysis reveals folate-dependent NADPH production. *Nature* 2014; 510(7504):298-302; PMID:24805240; <https://doi.org/10.1038/nature13236>
9. Xu IM, Lai RK, Lin SH, Tse AP, Chiu DK, Koh HY, Law CT, Wong CM, Cai Z, Wong CC, et al. Transketolase counteracts oxidative stress to drive cancer development. *Proc Natl Acad Sci U S A* 2016; 113(6):E725-34; PMID:26811478; <https://doi.org/10.1073/pnas.1508779113>
10. Lai RK, Xu IM, Chiu DK, Tse AP, Wei LL, Law CT, Lee D, Wong CM, Wong MP, Ng IO, et al. NDUFA4L2 fine-tunes oxidative stress in hepatocellular carcinoma. *Clin Cancer Res* 2016; 22(12):3105-17; PMID:26819450; <https://doi.org/10.1158/1078-0432.CCR-15-1987>