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RESEARCH HIGHLIGHT

Methionine restriction sensitizes cancer cells to immunotherapy

Weiqin Lu[1] | **Yongde Luo**[2]

[1]Department of Pharmaceutical Sciences, School of Pharmacy, University of Texas at El Paso, El Paso, USA

[2]School of Pharmacological Sciences & The First Affiliated Hospital, Wenzhou Medical University, Wenzhou, Zhejiang, P. R. China

Correspondence
Weiqin Lu, Department of Pharmaceutical Sciences, School of Pharmacy, The University of Texas at El Paso, 500 West University Ave., El Paso, TX 79968, USA.
Email: wlu2@utep.edu

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Altered metabolism is a defining characteristic of human cancer and holds significant diagnostic value in the clinic. A widely utilized diagnostic tool is the [^{18}F] deoxyglucose-positron emission tomography (FDG-PET) scan, which is based on the fact that cancer cells prefer to uptake glucose, a phenomenon known as the Warburg effect. In addition to the notable shifts in glucose metabolism, cancer cells exhibit distinct alterations in amino acid metabolism. Clinical studies have shown that PET imaging with [^{11}C]-methionine (Met-PET) provides stronger signals and higher specificity in tumor detection compared to the FDG-PET scan [1]. The dependence of cancer cells on exogenous methionine is referred to as the Hoffman effect [2], underscoring methionine addiction as a fundamental and general hallmark of cancer.

Methionine is a sulfur-containing essential amino acid and serves as a critical substrate in multiple metabolic pathways. One of its primary functions is in the synthesis of S-adenosylmethionine (SAM), a universal methyl donor that is essential for the methylation processes of DNA, RNA, lipids, histones, and other proteins. Following the donation of its methyl group, SAM is converted to S-adenosyl-homocysteine (SAH) and then homocysteine. Homocysteine can be recycled back into methionine in association with the folate cycle, which is fueled in large part by serine and glycine. Homocysteine can also be channeled into the transsulfuration pathway, where it is converted into cysteine for glutathione synthesis and redox homeostasis. Furthermore, methionine provides the methyl group for polyamine biosynthesis and can be recycled from methylthioadenosine, a by-product of polyamine biosynthesis, through the methionine salvage pathway (Figure 1). These interconnected biochemical pathways are intricately regulated to maintain metabolic balance and cellular function.

The addiction to exogenous methionine creates a metabolic vulnerability for cancer cells [3]. Numerous preclinical studies across various models have demonstrated that methionine restriction (MR) is highly effective against a wide range of cancer types and synergistic with chemotherapy [4]. Mechanistically, under MR conditions, cancer cells tend to selectively arrest in the late S/G2 phase

Abbreviations: cGAS-STING, Cyclic GMP-AMP synthase (cGAS)–stimulator of interferon genes (STING); FDG-PET, [18F] deoxyglucose-positron emission tomography; m^{6}A, N6-methyladenosine; Met-PET, [^{11}C]-methionine-positron emission tomography; MR, methionine restriction; PD-1, programmed death-1; PD-L1, PD-1 ligand; SAH, S-adenosyl-homocysteine; SAM, S-adenosylmethionine; VISTA, V-domain Ig suppressor of T cell activation; YTHDF1, YTH N6-methyladenosine RNA binding protein 1..

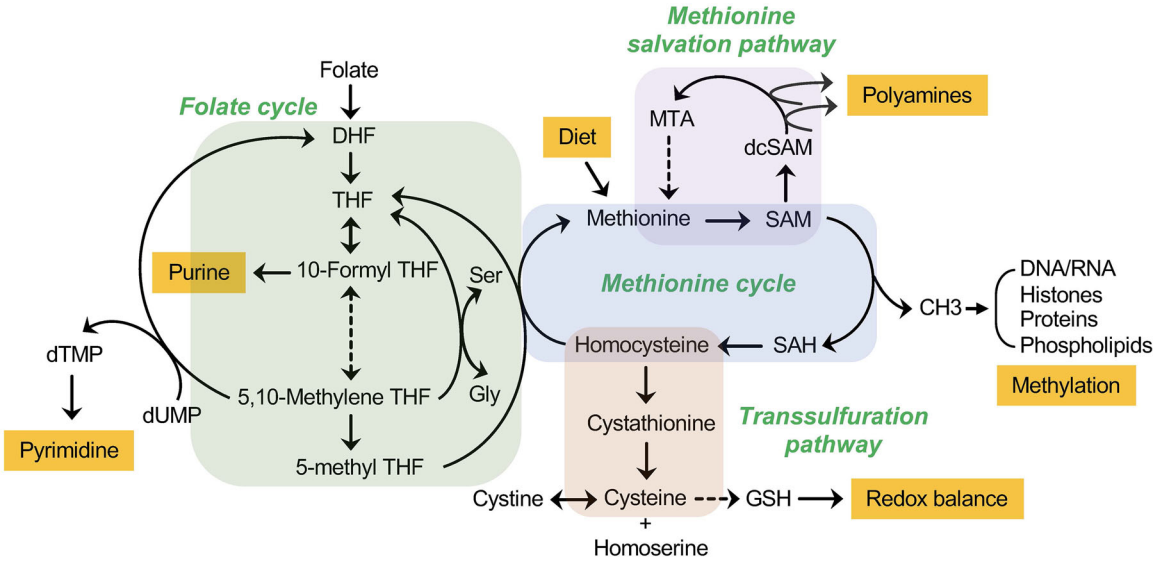

FIGURE 1 Methionine cycle and associated major metabolic pathways. The methionine metabolic cycle is intrinsically linked to several metabolic pathways, including the folate cycle, transsulfuration pathway, polyamine biosynthesis pathway, and methionine salvage pathway, forming an interconnected metabolic network. Therefore, methionine plays multifaceted roles in regulating the biosynthesis of purine and thymidine, amino acid homeostasis, polyamine synthesis, redox balance, as well as the methylation of DNA, RNA, lipids, histone, and other proteins. In addition, the recycling of homocysteine to methionine can also be driven by the betaine-derived methyl group in association with the choline oxidation pathway (not shown). Abbreviations: CH3, methyl group; dcSAM, decarboxylated S-adenosylmethionine; DHF, dihydrofolate; Gly, glycine; GSH, reduced glutathione; MTA, 5'-methylthioadenosine; SAH, S-adenosyl-homocysteine; Ser, serine; THF, tetrahydrofolate.

of the cell cycle [5]. This arrest effectively hinders cancer cell proliferation and creates a condition conducive to combining with chemotherapeutic agents that disrupt the cell cycle, ultimately enhancing cancer therapy. Recombinant methioninase, which depletes serum methionine after in vivo injection, has been developed as a cancer therapeutic agent that targets methionine addiction [6]. Importantly, recent studies showed that MR lowers the levels of SAM and the ratio of SAM/SAH, directly influencing the cellular methylation potential and epigenetic profile [5]. Furthermore, Bian et al. showed that tumor cells outcompete $CD8^+$ T cells for methionine by upregulating the expression of methionine transporter SLC43A2. This leads to the impairment of T cell immunity, indicating that methionine addiction in cancer cells may be an immune evasion mechanism [7]. However, the impact of MR on the epigenetic regulation of immune checkpoints, as well as its potential for sensitizing cancer cells to immunotherapeutic agents, remains elusive. These questions are essential for gaining a comprehensive understanding of how manipulating methionine metabolism may influence the interplay between cancer cells and the immune system and for exploring potential cancer therapeutic strategies involving MR in combination with immunotherapy.

A recent article published in *Gut* by Li et al. sheds light on a novel and crucial function of methionine metabolism in immune checkpoint regulation and anti-cancer immunity [8]. The study revealed that MR plays a pivotal role in restoring the infiltration of cytotoxic $CD8^+$ T cells and enhancing the efficacy of programmed death-1 (PD-1) blockade in colon cancer. Through a series of in vitro and in vivo models, the authors showed that methionine-derived SAM contributes a methyl group for METTL3/METTL14-mediated N6-methyladenosine (m^6A) methylation on the mRNAs that encode two critical immune-checkpoint proteins: PD-1 ligand (PD-L1) and V-domain Ig suppressor of T cell activation (VISTA). The reader protein YTH N6-methyladenosine RNA binding protein 1 (YTHDF1) recognizes the m^6A modification in the mRNAs encoding PD-L1 and VISTA, subsequently enhancing their translation efficiency. Elevated levels of YTHDF1 increase the synthesis of PD-L1 and VISTA proteins, leading to decreased levels of cytotoxic $CD8^+$ T cell infiltration into tumor nodules and, thus, increased levels of immune evasion. Conversely, MR, the knockdown of METTL3/METTL14, or the depletion of YTHDF1 reduces the synthesis of PD-L1 and VISTA proteins and increases the infiltration of functional $CD8^+$ T cells. This leads to the inhibition of tumor xenografts and chemically-induced colitis-associated colon cancer. Further, the authors demonstrated that MR or YTHDF1 deficiency synergizes with PD-1 blockade, resulting in increased levels of $CD8^+$ T cell infiltration, suppressed tumor growth, and extended overall survival. This research underscores the critical role of methionine as a methyl

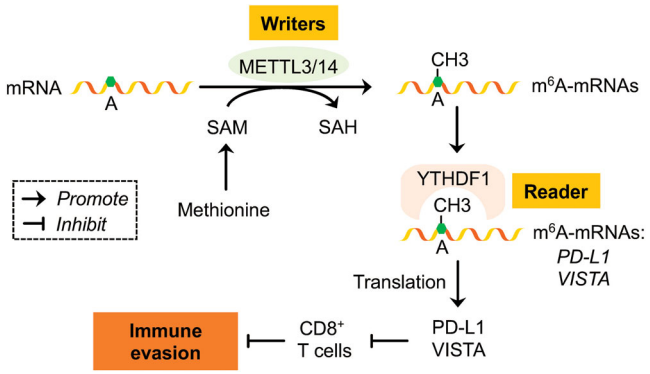

FIGURE 2 Methionine, methylation of PD-L1 and VISTA mRNAs, and antitumor immunity in colon cancer. Methionine serves as a substrate for the synthesis of SAM, a methyl donor essential for the methylation of N^6-adenosine residues in mRNAs. This process is catalyzed by the methyltransferase complex METTL3/14 (writer), resulting in the formation of m^6A-modified mRNAs. In colon cancer, the abundant m^6A reader protein YTHDF1 selectively binds to the m^6A-modified sites within PD-L1 and VISTA mRNAs, subsequently enhancing their translation. The heightened levels of these immune checkpoint proteins lead to the suppression of cytotoxic $CD8^+$ T cell infiltration, facilitating immune evasion. Conversely, dietary methionine restriction reduces the m^6A modification of PD-L1 and VISTA mRNAs, leading to a reduction in their subsequent translation. The reduction of these checkpoint proteins results in increased $CD8^+$ T cell infiltration and cancer regression. Abbreviations: A, adenosine; PD-L1, programmed death-1 ligand; SAH, S-adenosyl-homocysteine; SAM, S-adenosylmethionine; VISTA: V-domain Ig suppressor of T cell activation; YTHDF1, YTH N6-methyladenosine RNA binding protein 1.

donor in promoting the methylation of mRNAs of PD-L1 and VISTA, which limit the function of cytotoxic T cells and create an immune evasive microenvironment (Figure 2). In contrast, restriction of methionine promotes T cell immunity and subsequent tumor regression.

In conclusion, the research conducted by Li et al. has revealed the pivotal role of methionine in orchestrating cancer immunity through a novel epigenetic mechanism involving the methylation of mRNAs that encode critical immune checkpoint proteins essential for cancer immune evasion, offering new avenues for the development of innovative cancer immunotherapy. Recently, Fang et al. showed that MR promotes the activation of the cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) pathway and chromatin untethering through demethylation processes, ultimately enhancing the antitumor immune response [9]. These findings collectively suggest that the mechanistic link between MR and cancer immunity is complicated, likely encompassing a spectrum of genetic and epigenetic alterations. Additionally, studies have revealed the dependence of cancer cells on the exogenous supply of other amino acids, such as asparagine, glutamine, arginine, and serine [10]. Whether these amino acids directly contribute to immune evasion remains unclear. Nevertheless, given the multifaceted roles of methionine in purine and thymidine biosynthesis, amino acid homeostasis, epigenetic maintenance, and redox defense, further investigations into the intricate interplay of methionine metabolism in cancer, immunity, and drug resistance will be imperative for its future clinical applications.

DECLARATIONS

AUTHORS' CONTRIBUTIONS

Weiqin Lu and Yongde Luo wrote the manuscripts. Both authors read and approved the final manuscript.

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CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

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ORCID

Weiqin Lu https://orcid.org/0000-0002-6491-8203

References

1. Singhal T, Narayanan TK, Jacobs MP, Bal C, Mantil JC. 11C-methionine PET for grading and prognostication in gliomas: a comparison study with 18F-FDG PET and contrast enhancement on MRI. J Nucl Med. 2012;53(11):1709–15.
2. Hoffman RM, Erbe RW. High in vivo rates of methionine biosynthesis in transformed human and malignant rat cells auxotrophic for methionine. Proc Natl Acad Sci U S A. 1976;73(5):1523–27.

3. Sanderson SM, Gao X, Dai Z, Locasale JW. Methionine metabolism in health and cancer: a nexus of diet and precision medicine. Nat Rev Cancer. 2019;19(11):625–37.
4. Gao X, Sanderson SM, Dai Z, Reid MA, Cooper DE, Lu M, et al. Dietary methionine influences therapy in mouse cancer models and alters human metabolism. Nature. 2019;572(7769):397–401.
5. Coalson DW, Mecham JO, Stern PH, Hoffman RM. Reduced availability of endogenously synthesized methionine for S-adenosylmethionine formation in methionine-dependent cancer cells. Proc Natl Acad Sci U S A. 1982;79(14):4248–51.
6. Tan Y, Zavala J, sr., Han Q, Xu M, Sun X, Tan X, et al. Recombinant methioninase infusion reduces the biochemical endpoint of serum methionine with minimal toxicity in high-stage cancer patients. Anti-cancer Res. 1997;17(5B):3857–60.
7. Bian Y, Li W, Kremer DM, Sajjakulnukit P, Li S, Crespo J, et al. Cancer SLC43A2 alters T cell methionine metabolism and histone methylation. Nature. 2020;585(7824):277–82.
8. Li T, Tan YT, Chen YX, Zheng XJ, Wang W, Liao K, et al. Methionine deficiency facilitates antitumour immunity by altering m(6)A methylation of immune checkpoint transcripts. Gut. 2023;72(3):501–11.
9. Fang L, Hao Y, Yu H, Gu X, Peng Q, Zhuo H, et al. Methionine restriction promotes cGAS activation and chromatin untethering through demethylation to enhance antitumor immunity. Cancer Cell. 2023;41(6):1118–33 e12.
10. Butler M, van der Meer LT, van Leeuwen FN. Amino Acid Depletion Therapies: Starving Cancer Cells to Death. Trends Endocrinol Metab. 2021;32(6):367–81.

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