

## RESEARCH HIGHLIGHT

# Identification of immune-activating metabolite for enhancing T cell therapy of cancer

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**Funding information**

National Natural Science Foundation of China, Grant/Award Number: 81788101

T cell activation by antigens is followed by rapid growth, expansion, and differentiation to perform effector functions against infection or tumor. Upon antigen stimulation, T-cell receptor (TCR) signaling triggers the remodeling of metabolic activities to meet the demand of energy and nutrients for activating and launching effective immune responses [1]. Besides, the uptake and utilization of extracellular nutrients and metabolites is of great importance for regulating T cell activation and function [2]. Targeting metabolic activities, as well as the signals and metabolites transfer among immune cells and tumor cells, has acted an emerging part in cancer immunotherapy. Identifying metabolites to enable or disrupt T cell activation and function is helpful to understand T cell biology and design T cell immunotherapy.

A study by Wu *et al.* [3] published in *Nature Cell Biology* uncovered that asparagine (Asn), a non-essential amino acid in mammals secreted by tumor cells, is able

to enhance the TCR-mediated activation and efficacy of CD8<sup>+</sup> T cells towards tumor through lymphocyte-specific protein tyrosine kinase (LCK) signaling. This study provides insights into the physiological function of Asn in T cell activation and effector functions, showing the encouraging possibility of key metabolites for empowering cancer immunotherapy.

There are complicated communications among tumor cells and diverse immune cells, including the secretion and stimulation of cytokines, chemokines, and metabolites in the milieu, which modulate immune activation and surveillance, and in turn suppress or promote tumor progression [4]. Tumor cells and immune cells in the tumor microenvironment tend to take up different kinds of nutrients through cell-programmed signaling [5], implying the prospect to develop cancer therapies targeting the nutrient preference of cells. The roles of metabolites in regulating anti-tumor immunity attract much attention now. Another study from the same team demonstrated that tumor cells maintain high level expression of asparagine synthetase (ASNS) mediated by p53 and thus produce high level of Asn, which activates liver kinase B1 (LKB1)-adenosine 5'-monophosphate-activated protein kinase (AMPK) signaling for promoting tumor cell proliferation and survival [6]. Many types of tumor cells produce and secrete a large amount of Asn, but the physiological function of Asn on immune cells remains to be fully understood. Therefore,

**Abbreviations:** AMPK, adenosine 5'-monophosphate (AMP)-activated protein kinase; Arg, arginine; Asn, asparagine; ASNS, asparagine synthetase; Gln, glutamine; Glut-1, glucose transporter 1; GzmB, granzyme B; IFN- $\gamma$ , interferon gamma; LKB1, liver kinase B1; LCK, lymphocyte-specific protein tyrosine kinase; Leu, leucine; MDSCs, myeloid-derived suppressor cells; Met, methionine; mTORC1, mammalian target of rapamycin complex 1; PI3K, phosphatidylinositol 3-kinase; TCR, T cell receptor; TNF- $\alpha$ , tumor necrosis factor alpha; ZAP70, zeta chain of T cell receptor associated protein kinase 70

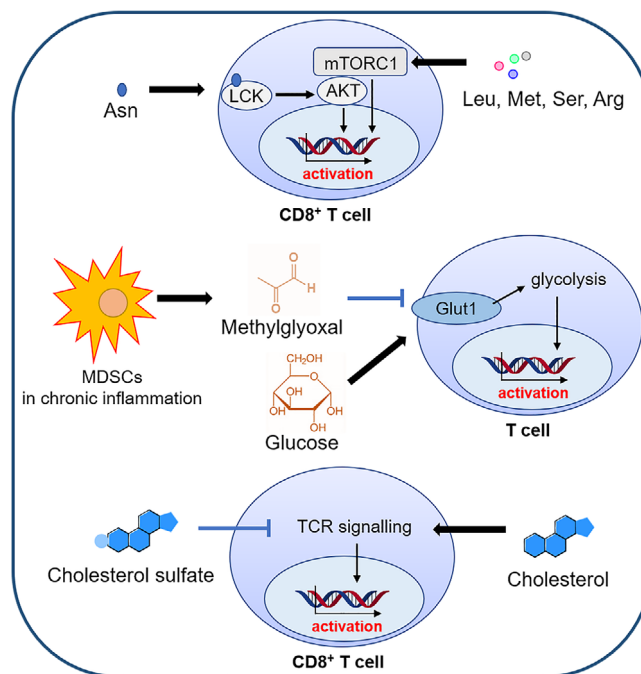
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Wu *et al.* [3] further addressed the concern and identified Asn as a positive regulator of CD8<sup>+</sup> T cell activation and function.

During the activation of naïve CD8<sup>+</sup> T cells stimulated with anti-CD3 and anti-CD28 antibodies, the addition of Asn upregulated the expression of surface activation markers (namely CD25, CD44, and CD69), the proportion of CD8<sup>+</sup> T cells with stemness feature (CD44<sup>+</sup>CD62L<sup>+</sup>), as well as the production of GzmB, IFN- $\gamma$ , and TNF- $\alpha$  [3]. Besides, Asn increased the apoptosis of cocultured B16-OVA melanoma cells, which supports that extracellular Asn strengthened the activation and function of CD8<sup>+</sup> T cells [3]. Apart from amino acids, the TCR-mediated uptake of glucose and the biosynthesis of cholesterol and cholesterol sulfates were reported to play important roles in T cell activation, subset differentiation, and effector functions [7]. Conversely, an active metabolite methylglyoxal secreted by myeloid-derived suppressor cells (MDSCs) was once revealed to be able to transfer into CD8<sup>+</sup> T cells, impair the uptake of glucose mediated by glucose transporter 1 (Glut-1), and ultimately prevent the activation of key kinases downstream of the TCR signaling [8]. However, lactate, instead of glucose, is necessary for tumor-infiltrating regulatory T cells to maintain their high suppressive function [9]. Collectively, these findings emphasized the roles of various metabolites in T cell activation and function (Figure 1).

Multiple amino acids, such as leucine (Leu), arginine (Arg), methionine (Met), glutamine (Gln), and Asn, were proved to promote the activation of mammalian target of rapamycin complex 1 (mTORC1) kinase [10–12]. Wu *et al.* [3] verified that Asn indeed increased the activity of mTORC1, but the Asn regulation of CD8<sup>+</sup> T cell activation was not deleted by the treatment of rapamycin (mTORC1 inhibitor), suggesting that mTORC1 activity was not likely to be sufficient for Asn-enhanced CD8<sup>+</sup> T cell activation. Consequently, Wu *et al.* [3] focused on LCK, a member of Src kinase family, recruited to TCR-CD3 complex to initiate antigen-specific TCR signalling through activating its downstream proteins, zeta chain of T cell receptor associated protein kinase 70 (ZAP70) and phosphatidylinositol 3-kinase (PI3K). Through equilibrium binding assay using <sup>3</sup>H-Asn and surface plasmon resonance assay (BIAcore), Asn was confirmed to directly bind LCK and regulate LCK activity, and the concentrations of Asn in naïve and activated T cells were sufficient for its binding with LCK. Specifically, Asn decreased Tyr505 phosphorylation of LCK, and promoted Tyr394 autophosphorylation in a dose-dependent manner. Therefore, Asn could be a physiologically relevant regulator of LCK activity, and LCK could be an intracellular sensor of Asn, leading to the signalling transduction in T cell activation and function.



**FIGURE 1** Metabolites in the regulation of T cell activation in anti-tumor immunity. Metabolites, including amino acids, glucose, and cholesterol, can influence TCR signaling, regulating T cell activation, differentiation, and anti-tumor responses. Upregulation of amino acid transporter SLC1A5 increases the uptake of Asn. Asn directly binds the intracellular sensor LCK, which initiates the antigen-specific TCR signaling, thereby enhancing CD8<sup>+</sup> T cell activation and effector functions in antitumor immune response. Abbreviations: Asn, asparagine; LCK, lymphocyte-specific protein tyrosine kinase; AKT/PKB, protein kinase B; mTORC1, mammalian target of rapamycin complex 1; Leu, leucine; Met, methionine; Arg, arginine; Ser, serine; MDSCs, myeloid-derived suppressor cells; Glut-1, glucose transporter 1; TCR, T cell receptor; SLC1A5, solute carrier family 1 member 5.

Cancer immunotherapy, such as immune checkpoint blockade and adoptive cellular therapy, is a powerful approach to treat cancer patients [13]. Amplifying the function of immune effector cells or diminishing the effect of immune-suppressive cells is the principal therapeutic strategy for now [14]. Wu *et al.* [3] performed adoptive T cell transfer assays to verify the influence of Asn on CD8<sup>+</sup> T cell responses *in vivo* towards infection and tumor. CD8<sup>+</sup> OT-1 T cells were adoptively transferred into CD45.2<sup>+</sup> mice with or without Asn feed in water, and then mice were infected with *Listeria monocytogenes*. Asn feed increased the amount of antigen-specific CD8<sup>+</sup> T cells and recall capacity *in vivo*. Likewise, intraperitoneal injection of Asn to mice on a Asn-deficient diet promoted antitumor CD8<sup>+</sup> T cell expansion and activation *in vivo*. Noticeably, tumor-bearing mice subjected to the adoptive transfer of CD8<sup>+</sup> cells that were activated for two days in Asn-containing

medium, showed reduced tumor metastasis and prolonged survival, supporting the fact that Asn treatment highly elevated the anti-tumor efficacy of CD8<sup>+</sup> T cells.

Collectively, the study by Wu et al. [3] demonstrated that the uptake of Asn in CD8<sup>+</sup> T cells enhanced the TCR-mediated activation and efficacy of CD8<sup>+</sup> T cells towards tumor and infection. Moreover, LCK was identified as a sensor of intracellular Asn, which initiated the antigen-specific TCR signaling. This study highlights the potential role of Asn in enhancing CD8<sup>+</sup> T cell activation and effector functions in adoptive transfer therapy. It is worth exploring whether Asn, or other metabolites, influence the exhaustion state of CD8<sup>+</sup> T cells. Also, whether Asn secreted by tumor cells could moderate the function of other immune cells, such as dendritic cells and tumor-associated macrophages, needs to be further investigated.

## DECLARATIONS

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF DATA AND MATERIALS

Not applicable.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## FUNDING

This work was supported by the grant from the National Natural Science Foundation of China (81788101).

## AUTHORS' CONTRIBUTIONS

B.C. drafted the manuscript. Z.C. made the figures. X.C. supervised and revised the manuscript. All authors read and approved the final manuscript.

## ACKNOWLEDGEMENTS

Not applicable.

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**How to cite this article:** Cong B, Cui Z, Cao X. Identification of immune-activating metabolite for enhancing T cell therapy of cancer. *Cancer Commun.* 2021;1-3. <https://doi.org/10.1002/cac2.12177>