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AUTHOR'S VIEWS



# Asparagine, a critical limiting metabolite during glutamine starvation

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**ABSTRACT**

A challenge of targeting glutamine metabolism in cancer is that tumor cells develop various strategies to adapt to glutamine limitation. We found that asparagine plays a critical role in supporting protein synthesis during glutamine starvation, highlighting a possible approach to optimize the therapeutic efficacy of targeting glutamine metabolism in cancer.

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Glutamine functions as a versatile donor of nitrogen and carbon atoms to support biosynthetic and bioenergetic reactions in proliferating cells.[1] However, glutamine levels in tumor environment are frequently found to be depleted under physiological conditions.[2] Thus, how tumor cells adapt to glutamine depletion becomes an important topic, particularly in term of developing novel therapeutic strategies that target glutamine uptake/metabolism. Along this line, we previously reported that exogenous supply of asparagine, a nonessential amino acid, is sufficient to rescue cell survival in tumor cells that undergo apoptosis during glutamine withdrawal.[3] Furthermore, this effect of rescue does not require restoring the tricarboxylic acid (TCA) cycle anaplerosis or other nonessential amino acids, both of which are routinely maintained via glutamine catabolism.[3] Since a chemical inhibitor of glutamine metabolism has entered clinical trials in cancer patients,[1] understanding the mechanism by which asparagine mediates cellular adaptation to glutamine limitation will be crucial to interpret the therapeutic responses.

In our recent study, we reported that the effect of asparagine to rescue glutamine deficiency is a generalizable phenomenon across a broad panel of tumor cell lines. In some cell lines, asparagine can even rescue proliferation defect during glutamine depletion.[4] Indeed, when exogenous glutamine is depleted, most mammalian cell lines are competent of synthesizing glutamine *de novo* to support the biosynthesis of nucleotides and nonessential amino acids, with the exception of asparagine. As a result, the reason that asparagine rescues cell proliferation during glutamine starvation is only due to the ability of asparagine to support protein synthesis (Fig. 1).[4] Unlike unicellular organisms, all tested mammalian cell lines lack cytosolic asparaginase activity to catabolize asparagine to fuel biosynthetic pathways. Interestingly, restoration of asparaginase activity in mammalian cells by using yeast or zebrafish orthologues can fully restore the capacity of mammalian cells to use asparagine as a biosynthetic substrate to support the TCA cycle anaplerosis, nucleotide biosynthesis, and even the synthesis of glutamine itself. However, under physiological levels of environmental asparagine, usually below 0.1 mM as it has been shown in human plasma, expression of zebrafish asparaginase (zASPG) suppresses cell growth and survival in glutamine-deficient medium *in vitro* and compromises xengraft tumor growth *in vivo*, which results from the depletion of intracellular asparagine. These results suggest that asparagine is a critical limiting metabolite during glutamine restriction, and lack of asparaginase activity may represent an evolutionary strategy that mammals use to adapt to pathophysiological variations of extracellular glutamine.

Glutamine metabolism has been extensively studied recently due to its versatile usage to support biosynthesis and bioenergetics beyond its role as an amino acid for protein synthesis.[2] Pioneer works showed that glutamine catabolism to glutamate and consequently to a-ketoglutarate is essential for glutamine-dependent cell growth and survival.[5,6] This is because a-ketoglutarate is a TCA cycle intermediate and glutamine-derived a-ketoglutarate fuels the TCA cycle to replenish the precursors that are consumed during various biosynthesis. Our results challenge this traditional paradigm by showing that asparagine can rescue glutamine-depletion-induced cell death or growth arrest without restoring the TCA cycle anaplerosis. Indeed,

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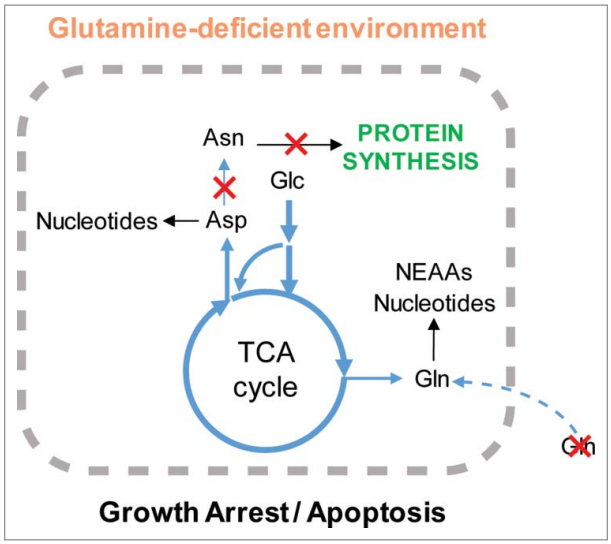


**Figure 1.** Key role for asparagine in protein synthesis during glutamine starvation. Most mammalian cells can maintain the TCA cycle anaplerosis and sustain glutamine biosynthesis *de novo* when environmental glutamine is restricted. However, asparagine biosynthesis is abolished under this condition, rendering cells to rely on exogenous supply of asparagine to support protein synthesis. Gln: glutamine; Asp: aspartate; Asn: asparagine; Glc: glucose; NEAA: nonessential amino acid; TCA: tricarboxylic acid.

most mammalian cells are competent to use glucose-derived carbon to fuel the TCA cycle when extracellular glutamine becomes limited, which is sufficient to drive the a-ketoglutarate efflux to support the biosynthesis of glutamate and glutamine. However, this efflux from the TCA cycle is not sufficient to drive the biosynthesis of asparagine, therefore leading to an inhibition of global protein synthesis. Notably, in addition to restoring intracellular asparagine, exogenous supply of asparagine dramatically induces the accumulation of glutamine synthetase (GLUL) protein at a post-transcriptional level. To demonstrate the critical role of glutamine biosynthesis, we used both pharmacological inhibition and CRISPR/Cas9-mediated genomic editing technology to confirm that GLUL is required for asparagine-dependent rescue of cell proliferation during glutamine starvation.

Recent works have also demonstrated a critical role of intracellular aspartate to support nucleotide biosynthesis.[7,8] Indeed, unicellular organisms can catabolize asparagine to produce aspartate through cytosolic asparaginase. But, we failed to detect meaningful conversion of asparagine to aspartate in all the tested mammalian cell lines, probably because of the lack of asparaginase activity.[4] In contrast, cell lines derived from fruit fly or zebrafish readily convert asparagine to aspartate even beyond the levels it is routinely maintained through glutamine catabolism. Interestingly, ectopic expression of two human orthologues of asparaginase did not confer meaningful asparaginase activity in the assays we tested. These findings imply that the asparaginase activity may be lost during late evolution, which can be co-evolved with the decrease of environmental asparagine levels. Therefore, asparagine is evolutionarily selected to be preserved rather than to be consumed for the production of aspartate, as the latter can be synthesized from the TCA cycle intermediate—oxaloacetate—via transamination (Fig. 1).

### *Therapeutic Outlook*

An inhibitor of glutaminase that converts glutamine to glutamate has entered clinical trials in cancer patients.[9] Our results suggest that tumor cells' response to glutaminase inhibitor may be dictated by environmental levels of asparagine. Along this line, bacteria-derived L-asparaginase has been used for decades to treat childhood lymphoblastic leukemia through depleting the circulating asparagine.[10] Thus, combination of the glutaminase inhibitor with L-asparaginase treatment may be a potential strategy to maximize the therapeutic efficacy. Furthermore, glutamine biosynthesis should also be explored as a potential therapeutic target, as our results indicate its indispensable role for tumor cell growth when exogenous glutamine is limited.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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