MOLECULAR & CELLULAR ONCOLOGY
2019, VOL. 6, NO. 5, e1536843 (3 pages)
https://doi.org/10.1080/23723556.2018.1536843

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AUTHOR'S VIEWS

Maintaining cytosolic aspartate levels is a major function of the TCA cycle in proliferating cells

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ABSTRACT

Cancer cells rely on glutamine to fuel mitochondria, however it remains unclear whether this is needed for bioenergetic or biosynthetic pathways. Our study suggests that an essential function of mitochondrial glutamine metabolism is to provide aspartate to the cytosol where it can be used for nucleotide and protein synthesis.

ARTICLE HISTORY
Received 11 September 2018
Revised 12 October 2018
Accepted 13 October 2018

KEYWORDS
Glutamine; CB-839; TCA cycle; aspartate; AGC1; aspartate-glutamate carrier; Aralar; Slc25a12; cancer metabolism; targeting metabolism; mitochondrial transporters

Background

Cancer cells acquire certain bioenergetic and biosynthetic demands. As the production of new proteins and organelles consumes great amounts of ATP, energetic needs of cancer cells are increased compared to non-proliferating cells. In addition to energy, cancer cells require building blocks such as nucleotides and amino acids to construct new cells.[1] A deficit of certain micromolecules could be a limitation for cell growth as much as the lack of energy. Therefore, the two major metabolic requirements for maintaining cell proliferation are production of sufficient energy and *de novo* synthesis or salvaging of micromolecules. Consequently, most cancer cells utilize glucose and glutamine as their main carbon sources.[2] Cancer cells commonly take up excessive amounts of glucose however convert almost every glucose molecule into lactate, even under normoxic conditions (Figure 1).[2] Due to this glycolytic phenotype, cancer cells were ascribed to have defective mitochondria and/or that mitochondrial dysfunctions may cause cancer. However, recent findings provide more evidence that despite prominent lactate secretion, mitochondria are still active in cancer cells and that mitochondrial function is essential for proliferation.[2-4] Along this line, cultured cancer cells use glutamine as the predominant carbon source to fuel mitochondrial tricarboxylic acid (TCA) cycle, known as glutamine anaplerosis (Figure 1).[2,5]

Glutamine replenishes the TCA cycle by being converted to glutamate via glutaminase enzyme and subsequently to alpha-ketoglutarate (α-KG). Several studies suggested that a decline in extracellular glutamine levels impairs cell proliferation and survival which led to development of glutaminase inhibitors with therapeutic potential.[5,6] Furthermore, pyruvate anaplerosis is known to improve the ability of cancer cells to tolerate glutamine starvation, suggesting that TCA cycle anaplerosis is essential for proliferation.[6] However, is it the bioenergetic or the biosynthetic reactions following glutamine anaplerosis are more limiting for proliferation remains unraveled. In our recent study, we found that an essential function of TCA cycle and glutamine anaplerosis in proliferating cells is to provide aspartate to the cytosol where it can be used for nucleotide and protein synthesis (Figure 1).[7]

Findings

Besides harboring bioenergetic pathways, mitochondria are also biosynthetic hubs for cancer cells by supporting *de novo* lipogenesis and nucleotide biosynthesis.[2] In addition, recent studies showed that mitochondrial redox homeostasis is crucial for maintaining cellular aspartate levels[3,4] which is described as one of the limiting metabolites for tumor growth, *in vivo*.[8,9] Hence, movements of *de novo* synthesized metabolites across mitochondrial membrane could impact cell proliferation.

To investigate the importance of mitochondrial aspartate export for proliferation, we first knocked-down mitochondrial aspartate-glutamate carrier 1 (AGC1; also known as ARALAR; gene name: Solute Carrier Family 25 Member 12 "*SLC25A12*").[7] AGC1 knockdown led to a significant drop in cytosolic aspartate levels and proliferation rate in several cell lines, suggesting that exporting aspartate from mitochondria is one of the requirements for cell proliferation.[7] More importantly, the proliferation defect of AGC1 knockdown (AGC1-KD) cells was strongly exacerbated when cells were cultured in a low-glutamine environment or when glutamine anaplerosis was inhibited using glutaminase inhibitor CB-839; arguing that inhibition of glutamine anaplerosis and mitochondrial aspartate export are synergistic.[7] Levels of cytosolic aspartate and the nucleotides downstream of aspartate were drastically declined upon glutaminase inhibition or during low-glutamine treatment and these levels, as well as the

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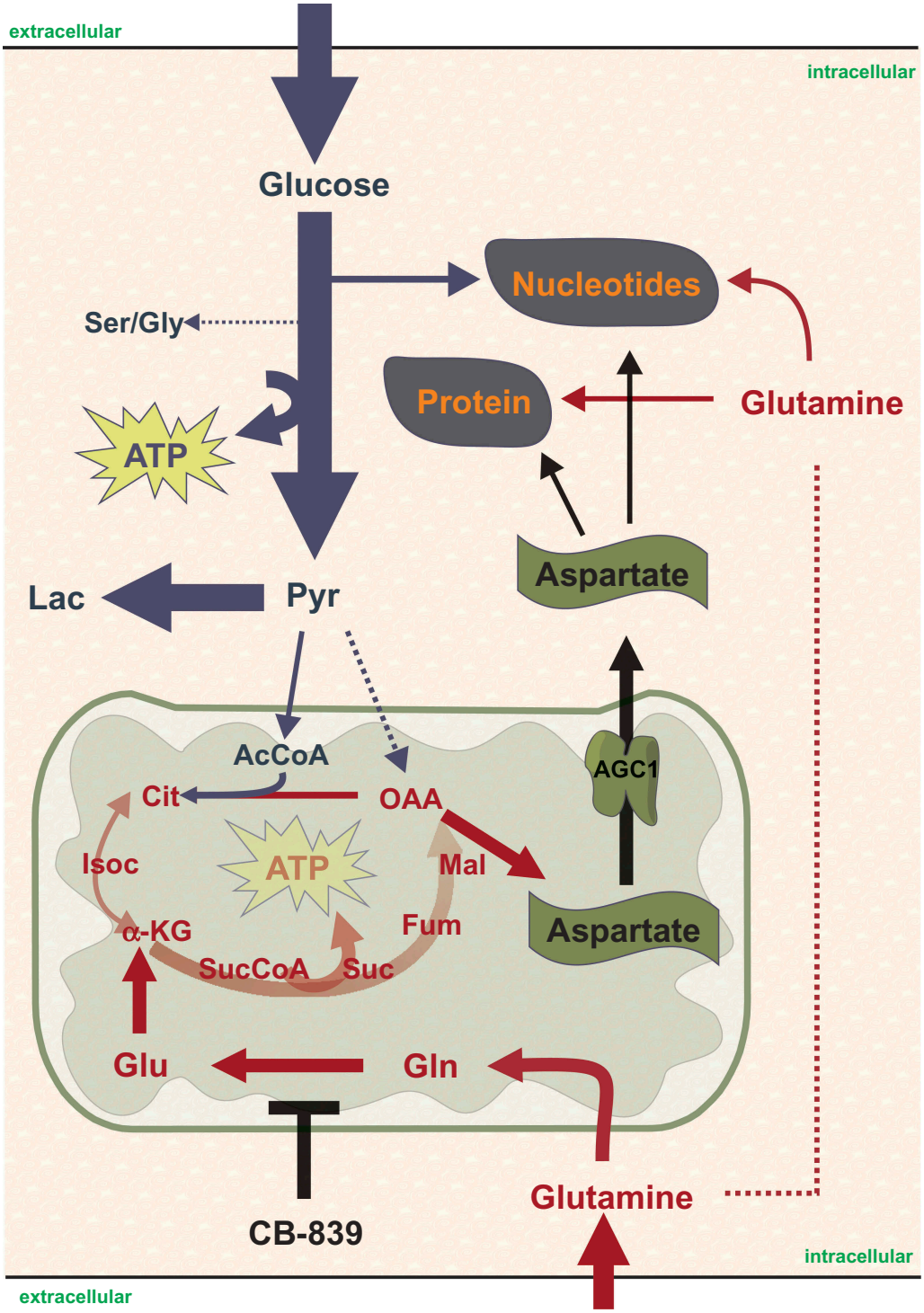

Figure 1. Pathways downstream of glucose and glutamine metabolism in proliferating cells.

While glucose is mostly converted to lactate, glutamine replenishes the TCA cycle to support aspartate biosynthesis. Aspartate delivery to cytosol becomes limiting for biosynthetic pathways and for cell proliferation/survival especially upon glutaminase inhibition with CB-839. Ser/Gly: serine/glycine; Pyr: pyruvate; Lac: lactate; AcCoA: acetyl-CoA; Cit: citrate; Isoc: isocitrate; α-KG: alpha-ketoglutarate; SucCoA: succinylCoa; Suc: succinate; Fum: fumarate; Mal: malate; OAA: oxaloacetate; Glu: glutamate; Gln: glutamine; ATP: adenosine triphosphate; AGC1: aspartate-glutamate carrier 1.

proliferation/survival rate of AGC1-KD cells were recovered by supplementation of exogenous aspartate.[7] Interestingly, inhibiting aspartate aminotransferases, which blocks aspartate anaplerosis, had no considerable impact on the ability of exogenous aspartate to rescue low-glutamine or CB-839 treatment, providing evidence that aspartate is not required to replenish TCA cycle for restoring proliferation.[7] These findings suggest that AGC1-KD cells suffer from a shortage of cytosolic aspartate in the absence of sufficient anaplerotic substrate, however could by-pass the need of TCA cycle function when sufficient exogenous aspartate is provided.[7]

Based on previous works,[10] we hypothesized that high levels of aspartate produced in mitochondria, subsequent to the TCA cycle, could potentially be exported by mitochondrial carriers other than AGC1 and some degree of proliferation could be maintained in the presence of sufficient glutamine anaplerosis. However, because the unspecific transporters may require higher K_m for aspartate, AGC1-KD cells cannot retain efficient cytosolic aspartate delivery when mitochondrial aspartate levels are declined after removal of glutamine, the major anaplerosis substrate. Confirming this, proliferation of AGC1-KD cells could also be rescued by supplementation of other anaplerosis sources such as pyruvate or dimethylalpha-ketoglutarate (a cell permeable form of α-KG) however only when aspartate aminotransferases are active. This suggests that TCA cycle function alone is insufficient to fully restore the proliferation when it is uncoupled from aspartate biosynthesis. Furthermore, because inhibition of mitochondrial respiration would also impair TCA cycle activity and reduce mitochondrial aspartate levels,[3,4] we investigated whether Complex I inhibition has similar impact on AGC1-KD cells as CB-839. Importantly, AGC1-KD cells were also more sensitive to Complex I inhibition compared to control cells, providing another evidence that the decline in mitochondrial aspartate levels was responsible for impaired cell growth and survival in AGC1-KD cells under glutamine limitations.

Finally, we reported that knocking down AGC1 in subcutaneously growing tumors significantly slowed-down tumor growth and increased their sensitivity to glutaminase inhibitor treatment. Because intracellular aspartate levels could limit tumor growth,[8,9] blocking cytosolic aspartate delivery (by inhibiting AGC1 or by mitochondrial respiration) could provide a novel strategy to sensitize CB-839-resistant tumors to the treatment in clinics.

Conclusions

In conclusion, our work provides evidence that a major function of TCA cycle in proliferating cells is to provide aspartate to cytosol which could be used for further biosynthetic pathways. Because aspartate supplementation is sufficient to maintain cell growth when TCA cycle is blocked, we argue that energy derived from mitochondrial respiration is not essential for proliferation and/or could be compensated through other pathways such as glycolysis. In addition, our work also underlines that local concentration (mitochondria) of a metabolite (aspartate) or abundance of its transporter (AGC1) could be a key determining factor for cell proliferation or survival under certain nutrient limitations (glutamine) which can be exploited for dual therapeutic possibilities to combat fast growing cancers.

Acknowledgments

We acknowledge Matthew Vander Heiden and his lab members for their generous helps during the creation of the original paper which this commentary is based on. We are also thankful for many other great research papers on this topic that we were unable to cite due to space limitations.

Funding

Bogner-Strauss lab is funded by the Austrian Science Fund FWF [SFB LIPTOX F3018 and P27108]; H.F.A trained within the doctoral program [W1226 DK] "Metabolic and Cardiovascular Disease" and was supported by Austrian Marshall Plan Scholarship 2017.

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