





Glutamine, MTOR and autophagy: a multiconnection relationship

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ABSTRACT

Cancer cells metabolize glutamine mostly through glutaminolysis, a metabolic pathway that activates MTORC1. The AMPK-MTORC1 signaling axis is a key regulator of cell growth and proliferation. Our recent investigation identified that the connection between glutamine and AMPK is not restricted to glutaminolysis. Rather, we demonstrated the crucial role of ASNS (asparagine synthetase (glutamine-hydrolyzing)) and the GABA shunt for the metabolic control of the AMPK-MTORC1 axis during glutamine sufficiency. Our results elucidated a metabolic network by which glutamine metabolism regulates the MTORC1-macroautophagy/autophagy pathway through two independent branches involving glutaminolysis and ASNS-GABA shunt.

Abbreviations: α KG: alpha-ketoglutarate; AMPK: AMP-activated protein kinase; ASNS: asparagine synthetase (glutamine-hydrolyzing); GLUD/GDH: glutamate dehydrogenase; GLS: glutaminase; GOT1: glutamic-oxaloacetic transaminase 1; MTORC1: mechanistic target of rapamycin kinase complex 1; TCA: tricarboxylic acid.

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Punctum

Cancer cells reprogram their metabolism to sustain their neoplastic proliferation and the necessary production of macromolecules. As they are often dependent on the amino acid glutamine, starving cancer cells to death has long ago been a potential therapeutic strategy against this disease. Our previous work demonstrated that glutaminolysis, the main metabolic pathway catabolizing glutamine into α -ketoglutarate (α KG), is sufficient to activate the master cell growth regulator MTORC1, thus inhibiting autophagy. However, until now the role of the bioenergetic status of the cell and its central controller AMPK in the glutamine-to-MTORC1 connection was never clarified.

Our data show that the activation of glutaminolysis is sufficient to increase the ATP levels and the subsequent inhibition of AMPK, suggesting that glutaminolysis affects the bioenergetic status of the cell [1]. This was not a surprise, because glutamine is responsible for 80% of the production of oxaloacetate through glutaminolysis, underscoring its active bioenergetic role. However, the connection between glutamine and the ATP-AMPK axis does not require glutaminolysis, as the inhibition of glutaminolysis does not affect the bioenergetic status of the cell. Strikingly, glutamine is sufficient to maintain the production of ATP, whereas leucine does not play any role other than allosterically activating GLUD (glutamate dehydrogenase). Under inhibition of glutaminolysis, the cells follow an alternative pathway involving the catalytic activity of ASNS (asparagine synthetase (glutamine-hydrolyzing)), GOT1, and the GABA shunt. All these

three elements have been connected previously to cancer metabolism and therapy resistance. However, this is the first time that this alternative pathway has been shown to mediate the connection between glutamine and MTORC1-autophagy signaling. Although in a different context, previous results already identified ASNS as a mediator of MTORC1 activation by the amino acid asparagine. Now, our results support a model by which ASNS metabolizes glutamine, and in combination with GOT1 and the GABA shunt, provides cells with an anaplerotic entry at the TCA cycle to produce oxaloacetate and the subsequent ATP synthesis in the absence of glutaminolysis. Indeed, we hypothesize that GLS (glutaminase) and ASNS can compensate for each other's activity when one or the other is inhibited, which could explain the lack of efficiency of strategies targeting GLS against cancer. This highlights the metabolic plasticity of cancer cells and the importance of glutamine in the maintenance of the metabolic homeostasis.

Although the role of leucine in MTORC1 activation has been extensively described, most of the leucine-induced activation mechanisms for MTORC1 signaling have been described following short-term approaches (10 min – 2 h). The accepted model for MTORC1 activation in response to amino acids assigns to leucine a major role in MTORC1 activation, with additional amino acids (such as glutamine and arginine) playing partial activation mechanisms. Now, our results indicate that leucine does not show sufficiency for MTORC1 activation in the long term, showing a permissive role for glutamine to be metabolized through

GDH for α KG production, as leucine is an allosteric activator of GDH. Still, our metabolomic, energetic, and signaling data indicate that leucine has almost no effect in cellular physiology in the absence of glutamine, severely questioning the currently accepted model placing leucine as the major signaling amino acid in terms of MTORC1 activation.

In our work, we also demonstrate the implication of the ATP-AMPK axis in the induction of glutamoptosis, i.e., the apoptotic cell death induced upon the inhibition of autophagy during nutritional imbalance. Indeed, reactivation of AMPK using metformin restores the autophagic flux and rescues the cell from this apoptotic phenotype during the metabolic imbalance. Treating mice bearing a colon cancer xenograft with DMKG (a cell permeable derivative of α KG), leads to an upregulation of MTORC1 activity and apoptosis within the tumor, that could be reverted using either temsirolimus (directly inhibiting MTORC1) or metformin (reactivating AMPK), both strategies that restore the autophagic flux. Thus, glutamoptosis can be triggered and reverted *in vivo*, with AMPK playing an active role in this process.

For a long time, MTORC1 has been known to be hyperactive in a large variety of human cancer types. Thus, different strategies to inhibit MTORC1 have been developed throughout the years. However, these therapeutic strategies fail to significantly improve the outcomes of patients. The failures of rapalogs in the clinic have been extensively explained due to the negative feedback loop downstream of MTORC1. This moved the pharmaceutical industry and academic research forward to develop dual inhibitors that can target both MTORC1 and MTORC2 to override this negative feedback loop. Counterintuitively, our work shows that these strategies of inhibiting MTORC1 or both MTORC1 and MTORC2 during nutritional imbalance would prevent glutamoptosis, thus promoting cancer cell survival. The high concentration of glutamine in the human serum, its high consumption by cancer cells, and the poor vascularization of solid tumors create a micro-environment that could meet the nutritional imbalance features, both poor in nutrients but also rich in glutamine. In this context, targeting MTORC1 would inhibit glutamoptosis, thus allowing cancer survival, which correlates with the cytostatic effect observed using rapalogs, not to mention the possible acquisition of rapamycin resistance phenotypes by the tumor cells. Glutamoptosis highlights a tumor suppressor feature of MTORC1 that was never described

before. This work contradicts the assumption regularly made that inhibiting a cell growth-promoting pathway will ultimately lead to tumor growth arrest.

From a therapeutic point of view, our results reinforce the idea of using co-treatments inhibiting autophagy and MTORC1 in cancer patients. Although therapeutic strategies targeting both MTOR signaling and autophagy are already under clinical investigation, a lack of efficient and specific inhibitors of autophagy exists. Our results showed that the upregulation of the autophagic receptor SQSTM1/p62 induces cell death during nutritional imbalance even when the cells are treated with rapamycin, thus suggesting an alternative co-treatment strategy to overcome the clinical limitations of rapalogs.

This work is the proof of concept of utilizing glutamoptosis as an Achilles's heel of cancer cells. Those tumors with higher avidity for glutamine or exposed to a micro-environment particularly rich in glutamine, are the best candidates to be sensitive to glutamoptosis in alternative therapeutic approaches.

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Reference

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