Metabolic impact of glutaminase isoenzymes modulation on glioma cell lines

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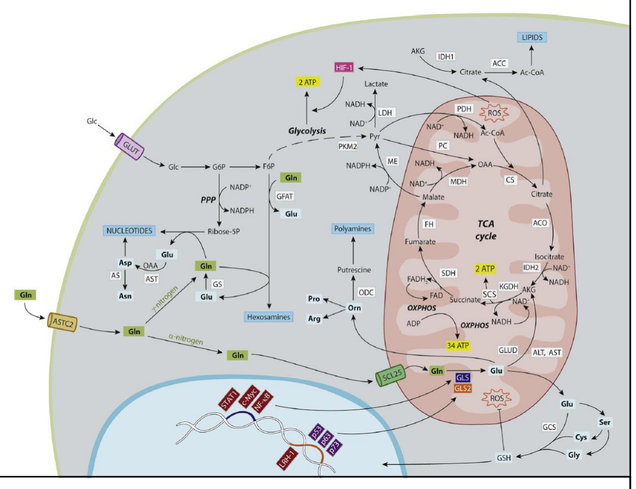
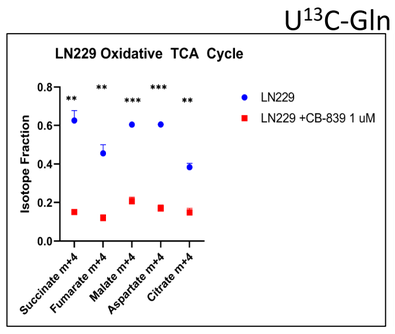
Many tumors use glutamine (Gln) for both energy generation and as a biosynthetic precursor. Glutaminases (GAs) catalyze the first step of glutaminolysis by converting Gln into glutamate and ammonia in the mitochondria. In humans, two genes encode for glutaminases: *GLS* and *GLS2*. *GLS* is widely considered as a tumor promoting gene and encodes two isoforms named KGA and GAC, and is usually overexpressed in many tumors. On the other hand, *GLS2* encodes isoforms GAB and LGA, and appears to have more complicated roles, including tumor-suppressive functions in some contexts. In glioma, *GLS2* is commonly silenced and *GLS* is usually overexpressed. We examined the metabolic consequences of inhibiting GLS activity in three glioma cell lines (LN229, T98G and U87MG) by using the clinically relevant inhibitor CB-839, or expressing GLS2, by generating a glioma cell model overexpressing GLS2 (LN229-GLS2), otherwise silenced. Both experimental conditions were analyzed by using a metabolomics approach for metabolite levels quantification in an Agilent Quadrupole Time of Flight LC-MS. We also performed stable isotope tracing experiments using U-^{13}C-labeled Gln and ^{15}N-labeled Gln in the amido group to ascertain the metabolic fates of Gln carbon and nitrogen.

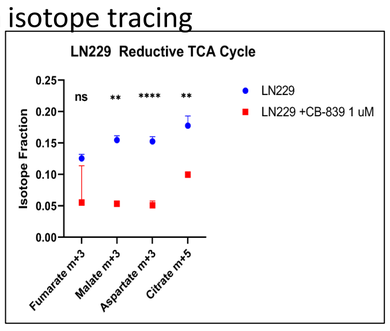


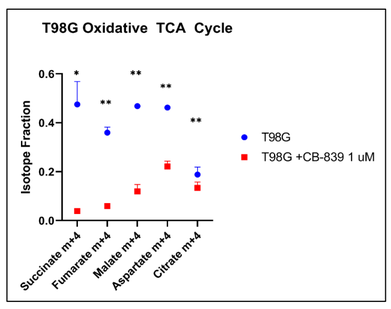
GLS inhibition by CB-839 in glioma cell lines

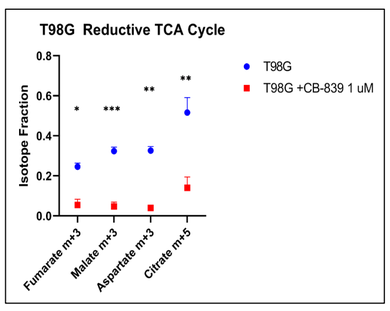
Tricarboxilic acid cycle related metabolites

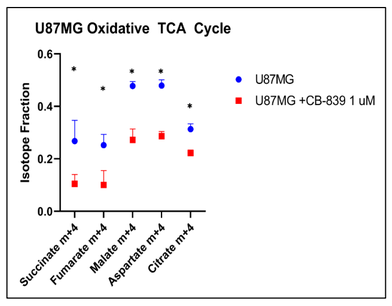
U^{13}C-Gln isotope tracing

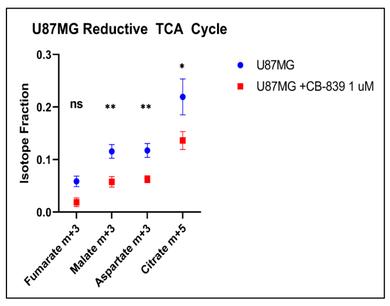




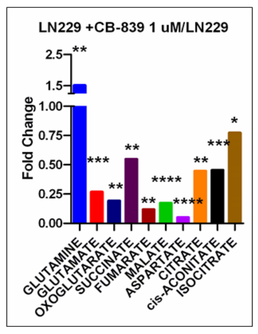


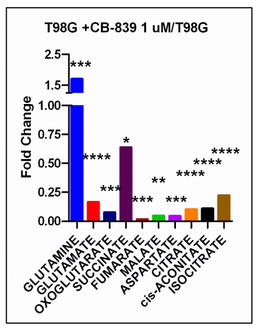


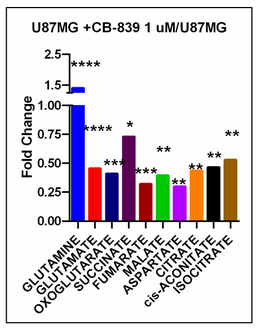




Metabolomics



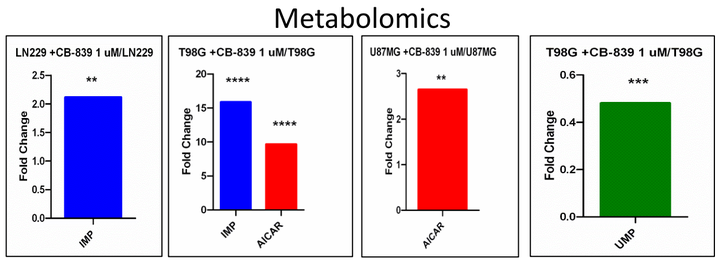


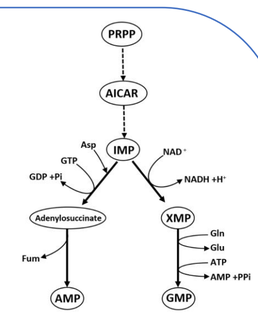


CB-839 reduces both oxidative and reductive TCAC metabolite labeling and metabolite levels

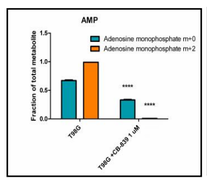
Nucleotide *de novo* biosynthesis

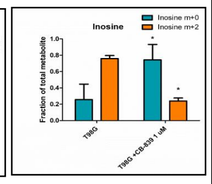
Metabolomics

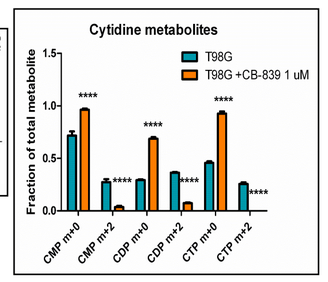


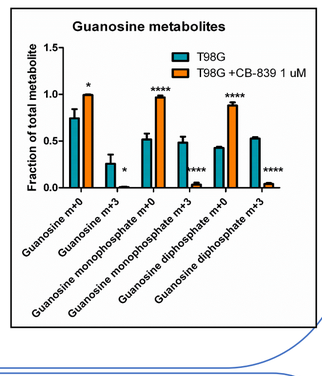


^{15}N(Amido)-Gln isotope tracing in T98G

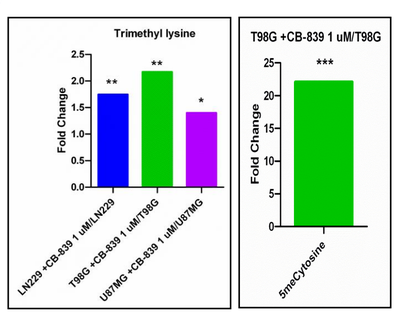


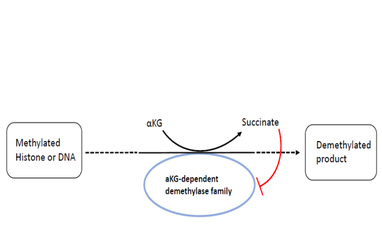


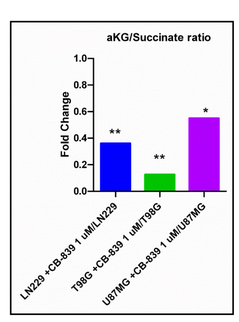




Changes in methylation patterns

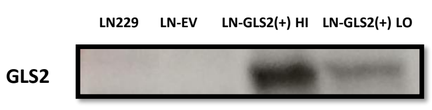


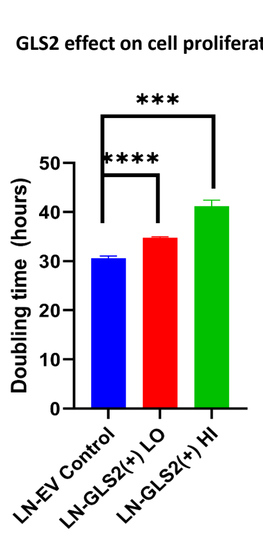


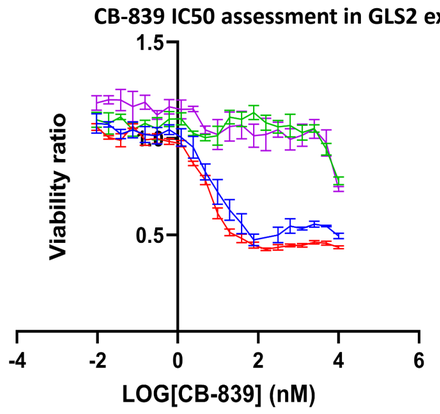


GLS2 overexpression models

We constructed two independent LN229 models stably transfected with a vector expressing human GLS2 at different levels, named LN-GLS2(+) HI and LO (higher and lower GLS2 expression levels), and an empty vector control, named LN-EV.

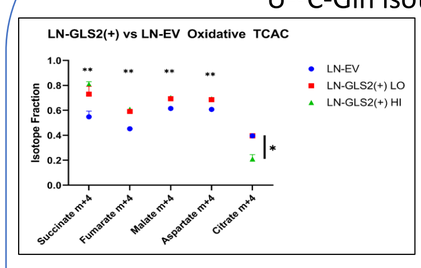


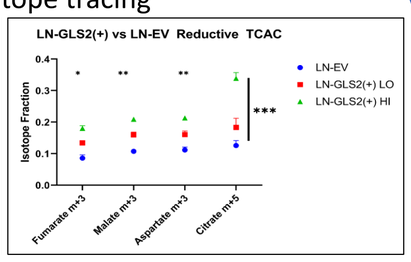




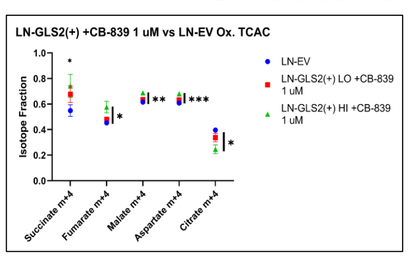
Tricarboxilic acid cycle related metabolites

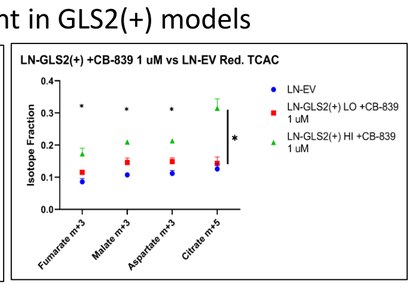
U^{13}C-Gln isotope tracing





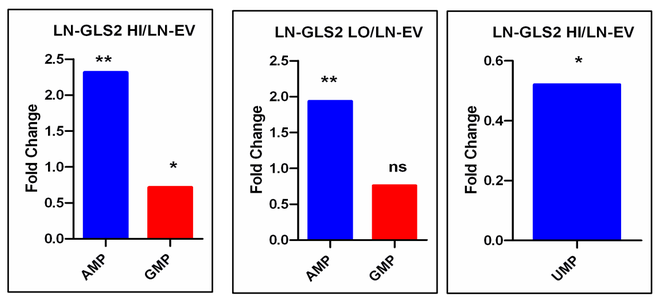
CB-839 treatment in GLS2(+) models





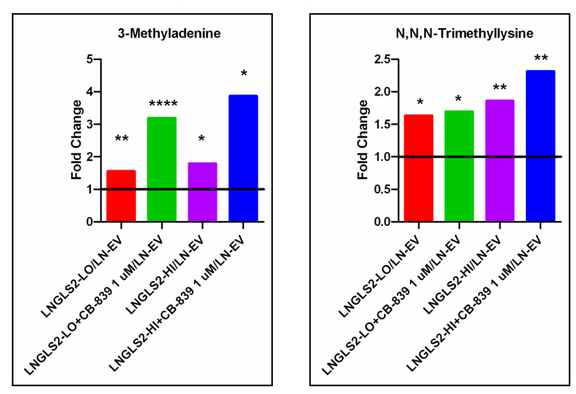
GLS2 overexpression increases both oxidative and reductive TCAC metabolites labeling in a dose-dependent manner, except for citrate, for which GLS2 expression apparently increases reductive labeling while decreasing the oxidative signature. When GLS selective inhibitor CB-839 is applied, GLS2 is able to rescue TCAC labeling from Gln.

Nucleotide *de novo* biosynthesis



GLS2 expression causes AMP accumulation while depleting GMP and UMP

Changes in methylation patterns



GLS2 expression increases the levels of methylated metabolites 3-methyladenine and trimethyl lysine, and concomitant GLS inhibition by CB-839 caused further accumulation of these metabolites.

Discussion

CB-839 treatment caused tricarboxilic acid cycle-related metabolites depletion in these glioma cell models as stated by metabolomics and U^{13}C-Gln tracing results. However, there were important quantitative differencies among the studied cell lines: T98G showed the greater effect, followed by LN229, and U87MG showed smaller differencies. This likely reflects differential dependencies of glioblastoma cells on GLS isoenzymes for maintaining levels of TCAC metabolites. We found reductive carboxylation-associated labeling in all GBM cell lines studied, so it may be of interest to further study the importance of this pathways in glioma. CB-839 apparently affected nucleotide *de novo* biosynthesis, causing accumulation of IMP and/or AICAR and depletion of UMP; using ^{15}N labeled Gln in the amido group, which functions as a nitrogen donor group for different pathways, we found a significant lower labeling in many nucleotides in T98G; this effect in nucleotides may be totally or partially explained due to aspartate depletion. CB-839 also appeared to increase methylation in nucleotides and proteins, noted by increased levels of 5-methyl-cytosine in T98G, and trimethyllysine in all three cell lines. These metabolites likely arise from degradation of methylated DNA and proteins, including histones. We speculate that these effects may be the result of a reduced activity of α-KG-dependent demethylases, which use α-KG as a substrate and can be inhibited by succinate or fumarate. We calculated the α-KG/succinate ratio and found that was significantly decreased in all three CB-839 treated cell lines. Regarding GLS2, its expression had an antiproliferative effect, noted by a dose-dependent increase in cell doubling time. We treated GLS2 expressing cells with GLS selective inhibitor CB-839 and calculated its IC50, and found that GLS2 was capable of rescuing cells from CB-839 effect on cell proliferation. We performed U^{13}C-Gln isotope tracing experiments in GLS2(+) models and found that GLS2 increased labeling of TCAC-related metabolites, but unexpectedly decreased oxidative labeling of citrate while increasing the reductive signature, so that we hypothesize GLS2 specific activities may cause a differential effect on anaplerosis compared to GLS. As expected, GLS2 expression maintained labeling levels when CB-839 was applied, again in a dose-dependent manner. GLS2 also showed to have an effect on nucleotide levels, likely related to nucleotide *de novo* biosynthesis, causing accumulation of AMP and depletion of GMP and UMP. Again, we speculate this may be the result of isoenzyme-specific activities of GLS2, since measured aspartate levels were higher with GLS2, so that it could not explain lower nucleotide levels. Lately, GLS2 caused accumulation of some methylated metabolites, that again likely arised from methylated DNA and proteins, such as 3-methyladenine and trimethyl lysine, following the same pattern of GLS inhibition. Even though, when we treated GLS2(+) models with CB-839 the levels of these methylated metabolites were further increased. In our opinion, all these changes, including similar effects caused by GLS inhibition and GLS2 expression, are the result of specific GLS2 roles that differentiate this isoenzyme from classic glutaminase GLS.

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