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Inhibition of Anaplerotic Glutaminolysis Underlies Selenite Toxicity in Human Lung Cancer

Supplemental Information

Inhibition of anaplerotic glutaminolysis underlies selenite toxicity in human lung cancer

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ABBREVIATIONS Non-Small Cell Lung Cancer (NSCLC); Glutaminase (GLS); Stable-isotope resolved metabolomics (SIRM); Cancer (CA); Non-cancerous (NC); Glutamine (Gln); Patient-derived (PD); Glutamate (Glu); heteronuclear single quantum coherence (HSQC); Ion chromatography-ultra high-resolution Fourier transform-MS (IC-UHR FT-MS); oxidized glutathione (GSSG); reduced glutathione (GSH); aspartic acid (Asp)

Figure S1. Selenite blocks the Krebs cycle activity in A549 cells. A549 cells were pre-incubated in [$^{13}C_6$]-glucose followed by treatment with vehicle or 6.25 μM Na_2SeO_3 for 0, 1.5, 3, 6, 10, and 24 h, extracted, and analyzed by GC-MS as described in Methods. The fate of carbon in glycolysis and the Krebs cycle is tracked with ● as ^{12}C (unlabeled) and ●, ●, ● as ^{13}C derived from PDH-, PCB-, and ME-mediated Krebs cycle reactions, respectively. The labeled patterns for the Krebs cycle result from the first turn except for those in brackets, which are derived from the second turn. Not all possible labeled patterns are shown. Shown in **A**-**H**, **L** (n=2) are the time course depletion of ^{13}C-metabolites by selenite. Also shown is time course inhibition of ^{13}C incorporation into proteinaceous Ala (**K**; n=1 or 2) and PCB protein expression (**I**-**J**; n=1 or 2) by selenite analyzed respectively by protein hydrolysis followed by GC-MS and Western blotting as decribed in Methods. ●, ●: ^{12}C and ■,■: $^{13}C_2$ in **A**, **C**, **E**; ●, ●: $^{13}C_1$ and ■,■: $^{13}C_3$ in **B**, **D**, **F**-**H**; ●, ●: Phe and ■,■: Val in **L**. In **J**, 1, 2, 5, 8-9, 12-13, 16-17 corresponded to vehicle at 0, 1.5, 3, 6, 11, and 24 h of treatment; 3-4, 6-7, 10-11, 14-15, and 18-19 were 6.25 μM selenite at 1.5, 3, 6, 11, and 24 h of treatment, respectively.

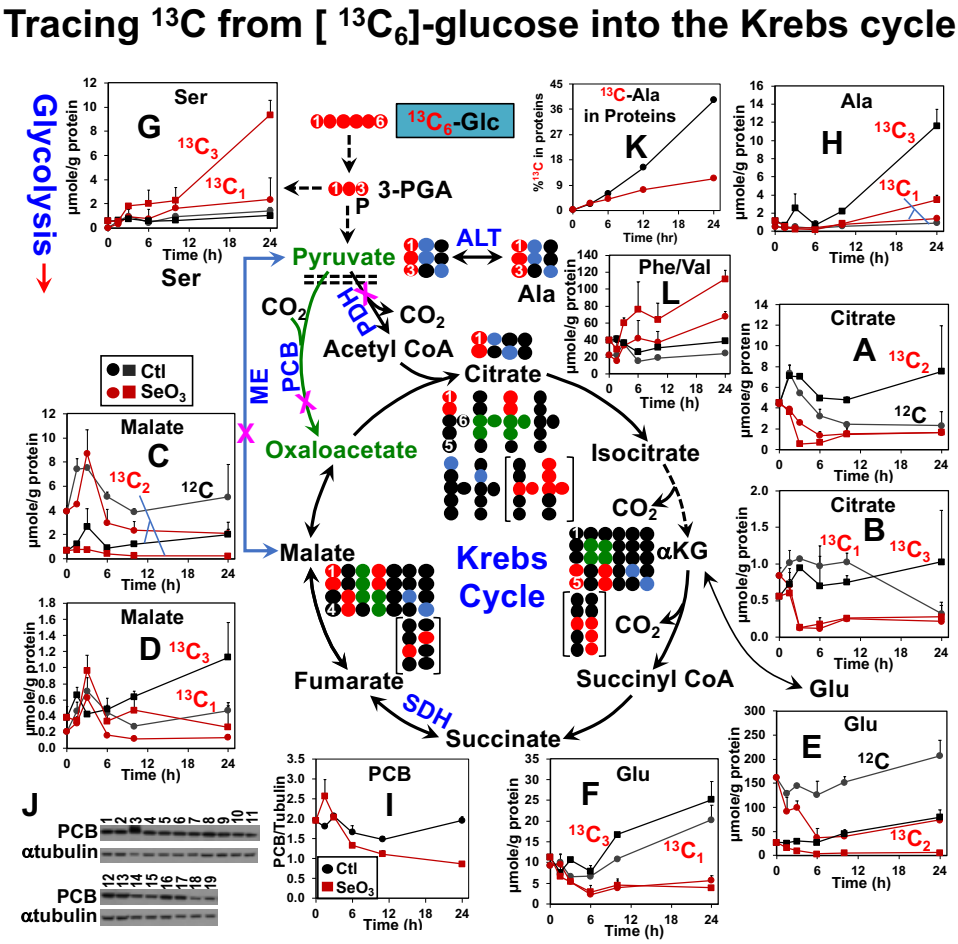

Figure S2. [$^{13}C_5$,$^{15}N_2$]-Gln tracer studies confirm disruption of pyruvate carboxylase and malic enzyme activities by selenite in A549 cells.

The [$^{13}C_5$,$^{15}N_2$]-Gln tracer-based time course experiment was performed similarly as in **Fig. S1** with sampling at 0, 1.5, 3, 6, 11, and 24 h and n=2. The fate of carbon in the Krebs cycle is tracked as in **Fig. S1**. The fate of carbon and nitrogen (◆ ^{14}N; ◆ ^{15}N) in glutaminolysis and Ser biosynthesis via gluconeogensis or GNG (●) is also traced. Not all possible labeled patterns are shown. Shown in **A**-**F** are the time course depletion of ^{13}C- or ^{13}C,^{15}N-metabolites by selenite that indicates compromised glutaminolysis and the Krebs cycle activity fueled by the ME and PCB reactions. Shown in **G** is the time course accumulation of ^{15}N- and ^{13}C,^{15}N-Ser induced by selenite, which requires GNG activity. The dramatic depletion of $^{15}N_1$-Ala by selenite in **H** suggests inhibition of Ala transaminase (ALT). ●, ●: $^{13}C_5$ and ■,■: $^{13}C_5$,$^{15}N_1$ in **A**; ●, ●: ^{12}C and ■,■: $^{13}C_4$ in **C**, **E**; ●, ●: $^{13}C_2$ and ■,■: $^{13}C_3$ in **B**, **D**, **F**; ●, ●: $^{15}N_1$ and ■,■: $^{13}C_1$,$^{15}N_1$ in **G**; ●, ●: ^{12}C,^{14}N and ■,■: $^{15}N_1$ in **H**. It should be noted that the GC-MS analysis employed in this experiment could not resolve ^{13}C from ^{15}N due to unit mass resolution. However, the m+6 species for Glu had to be $^{13}C_5$,$^{15}N_1$-Glu while the rest of the assignments were made in relation to independent UHR FT-MS analysis of representative A549 cell extracts (cf. **Figs. 5**, **S3**). For example, since $^{13}C_5$-Glu dominated over $^{13}C_4$,$^{15}N_1$-Glu in the FT-MS anaysis, the m+5 species was assinged to $^{13}C_5$-Glu in **A**.

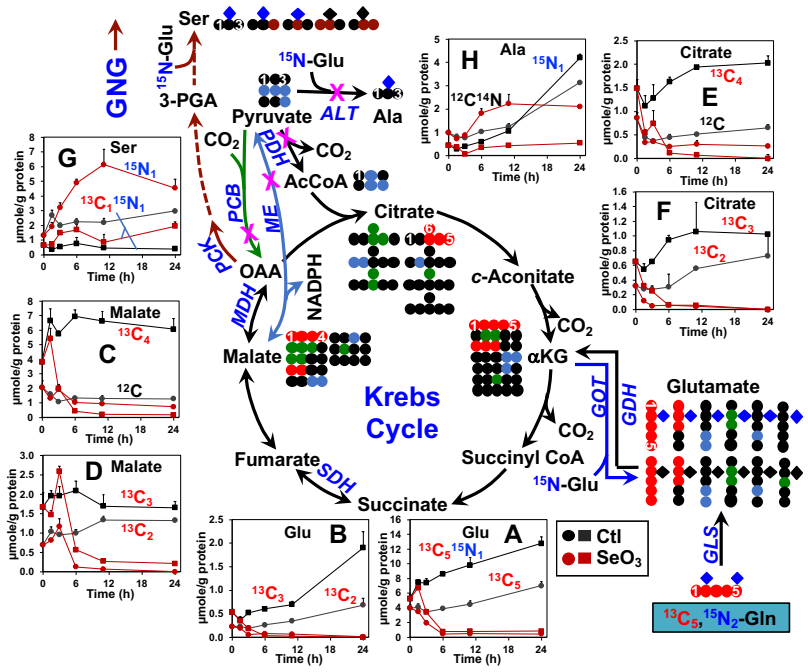

Figure S3. GLS1 inhibitor blocks glutaminolysis in both A549 cells and NSCLC patient tumor tissues.

A549 cells (**A**) were treated with 1 μM CB-839 or vehicle (DMSO) for 24-72 h. CA lung tissue slices of an NSCLC patient (**C**) were treated similarly for 24 h. Cells and tissues were extracted for polar metabolites, followed by analysis with 1D ^{13}C-filtered HSQC, as described in **Materials and Methods**. Shown are the protein weight-normalized HSQC spectra of 2 replicate extracts each of the control (Ctl) and CB-839-treated samples. Similar depletion of glutaminolytic products ^{13}C-Glu and -Asp were evident in 24-72 h of CB839-treated versus Ctl A549 cells. The plot in **B** displays the protein content of A549 cells after 24, 48, and 72 h of Ctl versus CB-839 treatments, which reflected reduced growth in CB839-treated cells. UXP: uracil nucleotides; other abbreviations are as in **Fig. 1**.

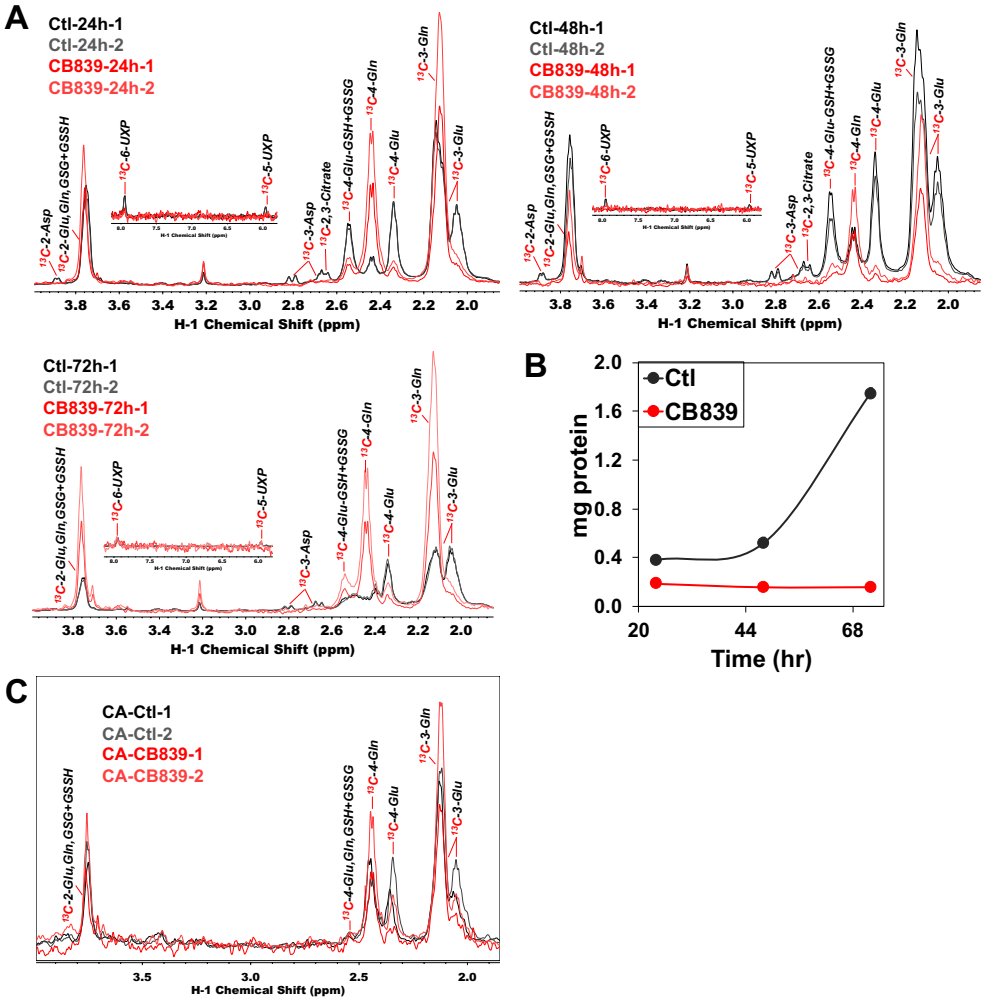

Figure S4. Glu supplementation fails to fully restore the Krebs cycle activity disrupted by selenite.

The data from **Fig. 5** were further analyzed for the Kreb cycle metabolites (**A**-**F**) and nucleotides (**G**-**H**) in terms of isotopologue distribution and total levels. $^{13}C_5$,$^{15}N_1$-Glu (*Glu) supplementation to A549 cells did not overcome the depletion in total, ^{13}C- and/or ^{15}N-isotopologues of fumarate, malate, citrate and Asp but enhanced the levels of total and ^{13}C- isotopologues of αKG and succinate in response to selenite. The selenite-induced reduction in isotopologue distribution and total levels for ATP or UTP were largely, if not fully restored by Glu supplementation. Black control, red + 5 mM Glu. * q<0.05, ** q<0.01,***q<0.001