Examination of the Role of Glutamine in Human Glioma Cell Metabolism with ^{13}C and ^{15}N NMR Spectroscopy

A. Mancuso[1,2], R. J. Deberardinis[1], and C. B. Thompson[1]

[1]Cancer Biology, University of Pennsylvania, Philadelphia, PA, United States, [2]Radiology, University of Pennsylvania, Philadelphia, PA, United States

Introduction:

In order for cancer cells to sustain a high rate of proliferation, they must produce fatty acids at a high rate for the synthesis of daughter cell membranes. A key step in this process is the production of cytosolic acetyl-CoA, which is catalyzed by ATP citrate lyase (ACL). Our laboratory has shown that ACL inhibition slows the growth of some cancer cells and could be an effective anti-cancer strategy (1). To improve our understanding of the consequences of ACL inhibition, we have been developing NMR methods to examine cancer cell metabolism in real time.

Previous studies in our lab have shown that glucose labeled in C-1 and C-6 can be used to monitor fatty acid synthesis (2). TCA cycle flux between citrate synthase and alpha ketoglutarate dehydrogenase was evident from the accumulation of label in C-4 of glutamate. However, complete TCA cycle activity, as indicated by labeling in C-2 and C-3 of glutamate was difficult to detect. We hypothesized that this was due to a significant unlabeled anaplerotic flux. To monitor changes in the rest of the TCA cycle and the malate shunt, additional sources of ^{13}C are necessary. In this work, we examined the use [3-^{13}C]glutamine alone and in combination with [1,6-$^{13}C_2$]glucose for monitoring TCA cycle, malate shunt, and fatty acid labeling. We also used [2-^{13}C]glucose to determine if any pyruvate entered the TCA cycle through pyruvate carboxylase.

Materials and Methods:

SF188 cells (human glioma grade 4, UC San Francisco) were grown in DMEM, supplemented with 10% FBS. They were perfused inside a 20-mm NMR tube while immobilized in a dense mass of porous collagen microcarriers (3). Continuous feed and product removal were used to maintain steady-state levels of nutrients (4). NMR spectra were acquired with a 9.4T spectrometer and a 20-mm liquids probe. ^{31}P spectra were used to determine total cell numbers; the parameters were: 60° pulse width, TR = 1000 ms, 4096 points, and 15000 Hz SW, 1200 excitations. The total viable cell number in the NMR tube, estimated from the NTP level (4), was $9x10^8$. ^{13}C spectral parameters were: 60° excitation, bi-level WALTZ16 ^1H decoupling, TR=1200 ms, 8192 points, SW=25000 Hz, 750 excitations, LB=3 Hz. For labeling experiments, either 10 mM [1,6-$^{13}C_2$] glucose or 4 mM [3-^{13}C] glutamine were used.

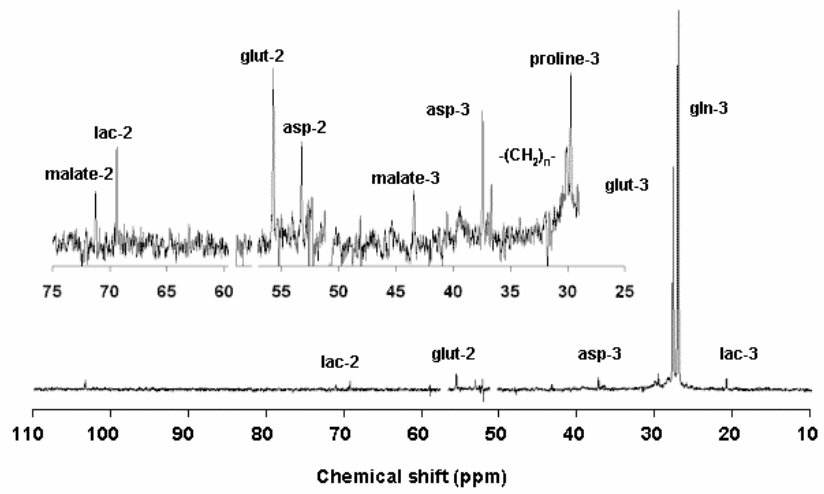
Results:

A typical spectrum obtained during perfusion with [3-^{13}C]glutamine is shown in the figure below. [3-^{13}C]glutamate was detected immediately and reached saturation within 1.5 hours. Cell extract studies with ^{15}N NMR demonstrated that a significant amount of glutamate was formed by glutaminase. Label at C-2 of glutamate, which requires deamination and complete TCA cycle activity (4), was not observed until 30 minutes after the addition of labeled glutamine. It reached saturation approximately 2.5 hours later. ^{15}N examination of extracts indicated that at least some alpha ketoglutarate was formed by alanine aminotransferase. Labeling in C-2 and C-3 of aspartate was detected within 15 minutes of the start of the experiment. The amount of label present at each carbon was comparable and both saturated within approximately 1 hour. Labeling in aspartate is believed to reflect labeling in oxaloacetate, since the two compounds are in rapid equilibrium. The equivalent labeling at the two central carbons supports the belief that label is distributed equally between C-2 and C-3 of succinate due to its symmetry. Label was also detected in C-2 and C-3 of malate, shortly after the addition of labeled glutamine. To our knowledge, this is the first report of a TCA cycle intermediate in cultured cells with real-time ^{13}C NMR spectroscopy. A small amount of label was detected in C-2 and C-3 of lactate, which demonstrates that four-carbon units from the TCA cycle are converted to pyruvate by either the pyruvate-citrate cycle associated with fatty acid synthesis, the malate shunt, or other similar pathways. Some labeled pyruvate apparently also re-entered the TCA cycle to form citrate since a low level of label was detected in fatty acids at 30 ppm. Cells perfused with both [3-^{13}C]glutamine and [1,6-$^{13}C_2$]glucose simultaneously, produced large amounts of [3,4-$^{13}C_2$]glutamate. This was very likely produced from [3,4-$^{13}C_2$]citrate, that was formed when [2-^{13}C]acetate (from glucose) combined with [2-^{13}C]oxaloacetate (from glutamine) by citrate synthase. With [2-^{13}C]glucose, which would strongly label C-2 of lactate by glycolysis, label was not detected in C-3 of glutamate. This would occur if pyruvate carboxylase were present, which would transfer label to C-2 of oxaloacetate and eventually to C-3 of citrate and glutamate.

Discussion:

Real-time ^{13}C NMR spectroscopy can provide unique information about metabolic pathways of cancer cells that would be difficult to obtain with any other non-invasive method. The results highlight some fundamental aspects of glioma cell metabolism that will serve as a reference for future studies with ATP citrate lyase inhibition. We are developing a ^{13}C kinetic model that will be used to estimate metabolic fluxes from the ^{13}C kinetic data. We expect that over the long run, these methods will prove to be powerful tools to facilitate the development of new approaches for the treatment of cancer.

Figure 1. Real-time ^{13}C spectrum of cells cultured with [3-^{13}C]glutamine.



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