Enhanced Kinetic Analysis of Hyperpolarized Pyruvate Metabolism in Cancer Cells Identifies a Compensatory Pathway Supplied by Glutamine

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Target Audience

Cancer researchers specializing in metabolic pathway analysis. Any researcher using hyperpolarized (HP) technology.

Purpose

Glutamine metabolism in cancer cells is highly versatile and can satisfy many of the energetic and biosynthetic demands of cell growth. In particular, because glutamine can be used to produce both alpha-ketoglutarate (AKG) and acetyl-CoA, it can enable cells to maintain Krebs cycle function even when glucose metabolism is impaired (1). Since a variety of pharmaceutical interventions target the glycolytic pathway, understanding the mechanisms by which cancer cells retain their viability under these treatments is essential for improving the efficacy of treatment regimens. In this study the impact of inhibiting mitochondrial pyruvate transport upon glutamine oxidationwas studied using HP [1-^{13}C]pyruvate in a glioblastoma cell line.

Methods

A solution of trityl radical OX063 (15 mM) and ProHance® ([Gd] = 1 mM) in [1-^{13}C]pyruvic acid was polarized in a HyperSense polarizer. SF188-derived glioblastoma cells overexpressing human Bcl-xl (SFxL) were grown on 15 cm plates in Dulbecco's Modified Eagle Medium (DMEM) modified to include 4 mM glutamine and no glucose. Two groups of cells were studied, the controls, and a group treated with UK5099, a well-known inhibitor of the mitochondrial pyruvate transporter. NMR experiments were carried out at 14.1 T using a Bruker CryoProbe. A 200 µL solution of HP [1-^{13}C]pyruvate was placed in the bottom of a 5 mm NMR tube. Upon insertion into the magnet, a suspension of 50 million SFxL cells was injected resulting in a final concentration of 6 mM pyruvate. Acquisition was queued, and spectra were acquired using hard 18^{o} inspection pulses with a 2 second T_r.

Results and Discussion

Injection of HP [1-^{13}C]pyruvate resulted in high quality ^{13}C spectra with detection of the metabolites [1-^{13}C]lactate, [1-^{13}C]alanine, and [^{13}C]bicarbonate with 2 s time resolution (Figure a,b). UK5099 quenched bicarbonate production by pyruvate dehydrogenase (PDH) and decreased alanine signal produced by alanine transaminase (ALT). Relative activities of the metabolites are shown in Figure c. Because suppression of PDH and ALT are predicted to impair availability of acetyl-CoA and AKG, we investigated whether glutamine metabolism was altered upon UK5099 treatment. Mass spectrometry and other experiments indicated that UK5099 enhanced ammonia secretion by activating glutamate dehydrogenase (GDH), thereby enabling AKG formation to persist even astransaminase activity decreased. Further metabolism of glutamine-derived AKG produced acetyl-CoA to maintain Krebs cycle function. A decreased alanine signal was not expected, as it is generally accepted that alanine aminotransferase (ALT) is primarily present in the cytosol. Estimates of the ratio of the cytosolic versus mitochondrial activities of ALT are ongoing. From a technological perspective, incorporation of the cryoprobe into the experimental protocol allows the detection of bicarbonate without having to resort to shaped excitation techniques previously using for cell studies. An experiment designed only to detect lactate and bicarbonate would obviously have missed the important information regarding alanine production. Furthermore, the number of cells used was halved, from 100 to 50 million, increasing throughput and decreasing costs.

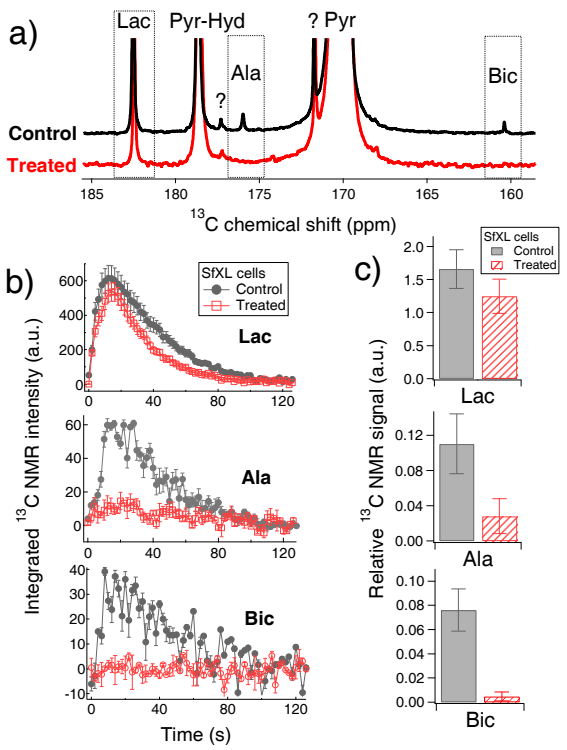

Figure: a) Representative hyperpolarized ^{13}C NMR spectra showing metabolic differences (Lac=lactate, Pyr-Hyd=pyruvate hydrate, Ala=alanine, Pyr=pyruvate, Bic=bicarbonate) between the control (top spectrum) and UK5099 inhibitor-treated SfXL cells (bottom spectrum) after administration of 6 mM hyperpolarized [1-^{13}C] sodium pyruvate to 50 million SfXL cells. The regions in the NMR spectra enclosed in dashed boxes highlight the metabolic signals that are affected by the treatment of UK5099 inhibitor in SfXL cells. b) Top to bottom: Kinetic timecourses of hyperpolarized ^{13}C NMR signals emanating from lactate, alanine, and bicarbonate for the control and UK5099-treated SfXL. c) Top to bottom: Comparative hyperpolarized ^{13}C NMR signals of lactate, alanine, and bicarbonate sum spectra for control and inhibitor-treated SfXL cells. The averaged NMR area for each metabolite was normalized relative to the NMR area of C2-pyruvate sum spectra. The error bars are standard deviations for *n*=3 trials.

Conclusions

Downregulated pyruvate metabolism is easily detected with a straightforward HP protocol. Lower carbohydrate oxidation in a glioblastoma cell line is compensated for by increased glutamine dependent metabolism facilitated by greater flux through GDH.

References

1. Yang C, Sudderth J, Dang T, Bachoo RG, McDonald JG, DeBerardinis RJ. Glioblastoma Cells Require Glutamate Dehydrogenase to Survive Impairments of Glucose Metabolism or Akt Signaling. Cancer Research. 2009;69(20):7986-93. doi: 10.1158/0008-5472.can-09-2266.