Analysis of Mitochondrial Metabolism in Cancer Cells by Combining Hyperpolarization and Isotopomer Analysis

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Introduction

Glycolysis and the formation of lactate (the Warburg effect) are well-known features of tumor metabolism. These activities occur in tumor cell lines as well as in live tumors. In culture, some tumor cells also use a variety of mitochondrial metabolic pathways to support bioenergetics and to provide precursors for cell growth.[1] While the Warburg effect can be evaluated *in vivo* using FDG-PET to monitor glucose uptake and MRS to assess lactate abundance, there are currently no techniques to monitor mitochondrial metabolism in tumors, or to compare the relative rates of glycolysis and mitochondrial fluxes. Because some aspects of mitochondrial metabolism are specifically linked to cell growth, there is considerable value in establishing techniques to image these activities. The transfer of hyperpolarized (HP) ^{13}C from pyruvate to lactate has been described in cancer models, but mitochondrial metabolism of pyruvate has not yet been observed in tumor cells. In this study, both the Warburg effect and mitochondrial metabolism were monitored with HP [1-^{13}C]-pyruvate in a glioblastoma cell line, SF188. Since HP $H^{13}CO_3^-$ could arise from two pathways, flux through pyruvate dehydrogenase (PDH) or pyruvate carboxylase (PC) and subsequent oxidation, we also analyzed fluxes by combining HP and thermally-polarized pyruvate joining a detailed steady-state metabolic analysis using ^{13}C isotopomer analysis along with the real-time kinetic analysis provided by the HP pyruvate on the same sample. This versatile method improves the accuracy of interpreting HP spectra and should be applicable to a variety of future labeling experiments.

Methods

SF188 glioblastoma cells were passaged in DMEM in 15-cm culture dishes as described.[2] On the day of the experiment, eight dishes of cells (200 million) were cultured for six hours in glucose-free DMEM supplemented with 6 mM Na-[3-^{13}C]-pyruvate. After this 'pre-incubation' period, the cells were trypsinized, rinsed and resuspended in 2.5 ml DMEM lacking glucose and pyruvate. 10.9 mg of [1-^{13}C]-pyruvic acid containing 15 mM trityl radical was hyperpolarized for 90 – 120 minutes prior to dissolution with 4 mL of 15.3 mM sodium bicarbonate. Following dissolution, 600 µl of the solution was placed in the bottom of a 10 mm NMR tube and inserted into the center of a Varian VNMRS 14.1 T system. The cell suspension was added via syringe at the start of acquisition. Serial 23-degree selective Gaussian pulses were applied every 2 seconds designed to excite [1-^{13}C]-lactate and [^{13}C]-bicarbonate simultaneously over 3 – 4 minutes. Following acquisition of the HP signals, the cells were immediately harvested, frozen, and extracted with 12% perchloric acid. Acid-soluble metabolites were lyophilized and analyzed by NMR spectroscopy as described[2] for isotopomer analysis.

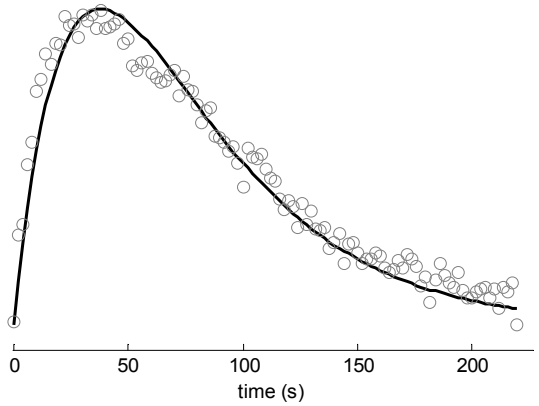

Figure 1: [1-^{13}C]-Lactate HP signal evolution following addition of 6 mM HP [1-^{13}C]-pyruvate. The line through the data represents a two-pool exchange model for C13 into the lactate pool.

Results

Following the addition of HP [1-^{13}C]-pyruvate, [1-^{13}C]-lactate was immediately detected and a kinetic time-course can be monitored (figure 1). Preliminary kinetic modeling of the lactate signal resulted in a pyruvate-to-lactate flux of 0.046 µmol/hr/billion cells. [^{13}C]-Bicarbonate was observed in a summation of spectra (figure 2) while [1-^{13}C]-alanine could be detected with a non-selective pulse (data not shown). Interestingly, HP data resulted in a large lactate signal and relatively small alanine signal, while spectra of extracted material showed the opposite result. A small amount of [^{13}C]-bicarbonate was also present in the extracted material. Based on previous analyses after incubating these cells with ^{13}C-labeled glucose[3], we predicted that the [^{13}C]-bicarbonate arose through the direct activity of PDH on [1-^{13}C]-pyruvate. The other possible pathway to hyperpolarized $H^{13}CO_3^-$ would be entry of [1-^{13}C]-pyruvate into the TCA cycle via PC followed by generation of $H^{13}CO_3^-$ after a partial turn of the cycle. The HP experiment cannot differentiate between these two pathways. However, since the cells were exposed to [3-^{13}C]-pyruvate before the HP experiment, an isotopomer analysis of the cell extract should provide this information. The ^{13}C spectrum of the extract showed highly enriched glutamate C2, C3 (Fig. 2 inset) and C4 and the isotopomer analysis of those relative multiplet areas gave the relative activities of PDH and PC. This analysis showed that the majority of the acetyl CoA pool was ^{13}C enriched, consistent with a high PDH activity, and anaplerotic flux was also high but very little resulted from carboxylation of pyruvate (PC).

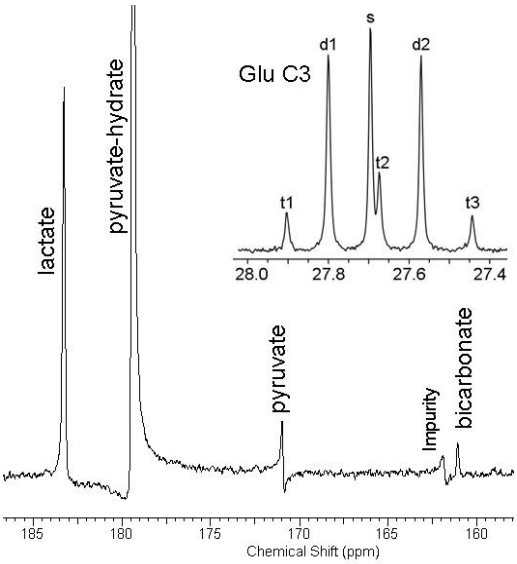

Figure 2: ^{13}C HP spectrum. Sum of 30 fids using 23° double-Gaussian selective excitation. Shaped pulses caused the phasing errors visible in this spectrum. [1-^{13}C]-Pyruvate is referenced to 171.1 ppm. Both [1-^{13}C]-lactate (183.4 ppm) and $H^{13}CO_3^-$ (161.2 ppm) are detected as well as [1-^{13}C]-pyruvate hydrate (179.5 ppm) and a sample impurity at 162.1 ppm. The inset shows the glutamate C3 multiplet of metabolites extracted from the same cells.

Conclusions

The use of selective pulses allows enhanced signal of down-stream metabolites like lactate and bicarbonate as well as dramatically reducing the strong pyruvate signal from the spectrum. The appearance of bicarbonate in the HP spectra was due to flux through PDH rather than PC. The relatively low percentage of label in the lactate pool demonstrates a profound difference between pyruvate metabolism in cells provided with glucose and cells given pyruvate in the absence of glucose.

REFERENCES

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