HYPERPOLARIZED [U-2H, U-13C]GLUCOSE REPORTS ON GLYCOLYTIC AND PENTOSE PHOSPHATE PATHWAY ACTIVITY IN EL4 TUMORS AND GLYCOLYTIC ACTIVITY IN YEAST CELLS.

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Target audience

The data will concern researchers in the field of dissolution DNP, carbon-13 spectroscopy and those interested in cancer metabolism.

Purpose

^{13}C magnetic resonance spectroscopic imaging measurements of the flux of hyperpolarized ^{13}C label between [U-^{2}H, U-^{13}C]glucose and lactate has been used recently to image glycolytic flux in tumors[1]. In addition to signals from glucose and lactate several other resonances were observed from metabolic intermediates, including a signal that was tentatively assigned to 6-phosphogluconate (6PG) on the basis of similar studies in yeast cells[2]. Since 6PG is an intermediate in the pentose phosphate pathway (PPP) this raised the possibility that flux could also be measured in this pathway. However, the resonance of 6PG is very close to that of 3-phosphoglycerate (3PG), an intermediate in the lower part of glycolysis, and in previous work on breast cancer cells *in vitro* this signal was assigned to 3PG[3]. We sought to resolve this discrepancy in peak assignment by measuring glycolytic and PPP flux using a liquid chromatography - mass spectrometry (LC/MS-MS) approach.

Methods

Chemical shift standards: A range of glycolytic and PPP intermediates (100 mM) were dissolved in 20 mM phosphate buffered $^{2}H_2O$ at pH 7 and carbon-13 MR spectra were acquired on an 11.7 T magnet (Bruker) at 300 K. ***LC/MS-MS:*** EL4 tumor-bearing mice were injected with 0.2 mL of a 200 mM [U-^{13}C]glucose solution and tumors were excised and freeze-clamped after 60 s with liquid nitrogen cooled tongs. Yeast was incubated in a 4 mM [U-^{13}C]glucose solution and samples were quenched in ice-cold methanol after 60 s. Tumors and yeast were extracted with acetonitrile:methanol 75:25 with 0.2% formic acid. Metabolites were analyzed using an Infinity UPLC system (Agilent 1290) and a triple quadrupole mass spectrometer (Agilent 6460) as described pereviously[4]. ***Hyperpolarized [U-^{2}H, U-^{13}C]glucose in yeast cell suspensions*:** [U-^{2}H, U-^{13}C]glucose was polarized and dissolved as described previously[2]. 120 ^{13}C spectra (nt=4, TR=0.5s) of wild-type and *zwf1*Δ yeast were acquired on a 9.4 T spectrometer (Varian) at 30 °C.

Results

The chemical shifts for 3PG and 6PG were 181.04 ppm and 181.64 ppm, respectively. In EL4 tumors both the 6PG and 2/3PG pools became labeled rapidly following injection of ^{13}C-labelled glucose (Figure 1), the degree of labeling reflecting the relative pool sizes. LC-MS cannot differentiate between 2PG and 3PG. Tumor ^{13}C-6PG was more abundant than ^{13}C-2PG/3PG and the resonance at ~181 ppm in *in vivo* spectra of EL4 tumor-bearing mice injected with hyperpolarized [U-^{2}H, U-^{13}C]glucose matched more closely that of 6PG. ^{13}C MR spectra of wild-type and *zwf1*Δ yeast cells showed a resonance at ~181 ppm after labeling with hyperpolarized [U-^{2}H, U-^{13}C]glucose, however there was no 6PG in *zwf1*Δ cells as shown by the LC/MS-MS analysis. In the wild-type cells 3PG was approximately four-fold more abundant than 6PG.

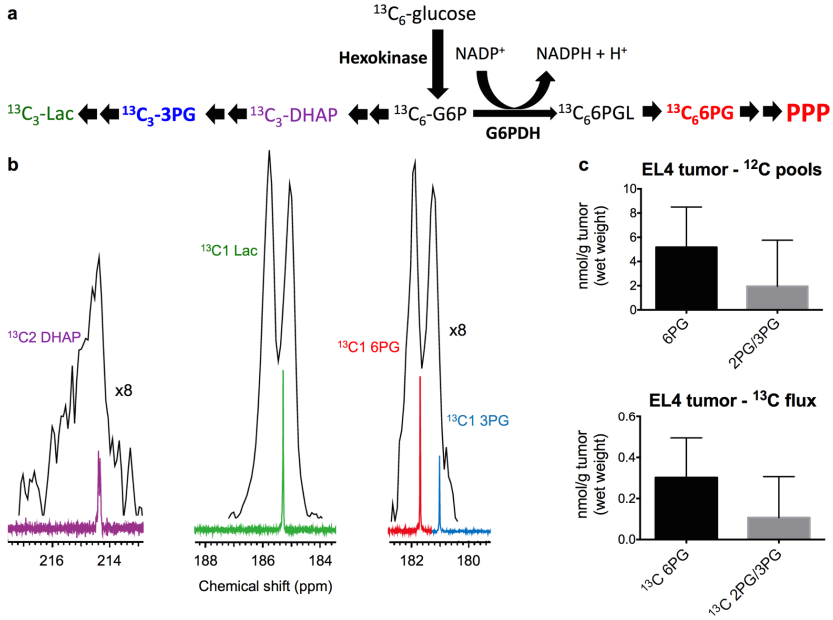

Figure 1 a) Glucose metabolism in the glycolytic and the pentose phosphate pathways. Dihydroxyacetone phosphate (DHAP), 3-phosphoglycerate (3PG), lactate (Lac), 6-phosphogluconolactone (6PGL), 6-phosphogluconate (6PG). b) Spectra of the ^{13}C chemical shift standards acquired at 125 MHz (300 K, 16 k transients) referenced to an internal TMS standard: C2 DHAP (pink) at 214.37 ppm, C1 Lac (green) at 185.33 ppm, C1 6PG (red) at 181.64 ppm and C1 3PG (blue) at 181.04 ppm. Representative spectrum (black) from an EL4 tumor in an animal injected with hyperpolarized [U-^{2}H, U-^{13}C]glucose reproduced from reference (3) with permission. The spectrum is referenced to the DHAP resonance at 214.37 ppm. (c) LC/MS-MS measurements of the concentrations of ^{12}C and ^{13}C labeled 6PG and 2PG/3PG in EL4 tumors (n=5) 60 s after injection of 200 mM $^{13}C_6$ glucose.

Discussion

Overlay of hyperpolarized ^{13}C spectra from EL4 tumors *in vivo* on spectra of chemical shift standards shows that the resonance at ~181 ppm corresponds to 6PG. The LC/MS-MS data show that at 60 s after injection of ^{13}C-labelled glucose into EL4 tumor bearing mice there is ~2x more labeled 6PG as 2/3PG, supporting the assignment. PPP flux is increased in oxidatively stressed cancer cells in order to increase antioxidant power[5,6]. This may allow hyperpolarized [U-^{2}H, U-^{13}C]glucose to be used for real-time measurements of PPP flux in tumors *in vivo* and hence their capacity to resist oxidative stress. In wild-type yeast there was ~4x more 2/3PG as 6PG and no 6PG in G6PDH deficient zwf1 mutants, which shows conclusively that the resonance corresponds to 3PG in yeast. Flux measurements of PPP are therefore not possible in yeast with hyperpolarized [U-^{13}C, U-$^{2}H_2$]glucose.

Conclusions

The resonance at ~181 ppm in ^{13}C spectra of EL4 tumors following injection of hyperpolarized [U-^{2}H, U-^{13}C]glucose is predominantly from 6PG, an intermediate in the PPP. Measurements of this resonance may allow assessment of changes in PPP flux in tumors. In yeast cells the resonance at ~181 ppm is predominantly from 3PG, which together with the resonances from pyruvate, DHAP and ethanol may provide further insight into fluxes in various parts of the glycolytic pathway.

References

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