Monitoring PKM2 status in glioblastoma using hyperpolarized ^{13}C MRS

Myriam Marianne Chaumeil[1], Joydeep Mukherjee[2], Humsa Venkatesh[1], Russell O Pieper[2], and Sabrina M Ronen[1]

[1]*Radiology, University of California, San Francisco, San Francisco, CA, United States,* [2]*Neurological Surgery, University of California, San Francisco, San Francisco, CA, United States*

INTRODUCTION

Pyruvate kinase (PK) is the glycolytic enzyme which catalyzes the dephosphorylation of phophoenolpyruvate (PEP) to pyruvate. Whereas normal cells express the M1 isoform of PK, cancer cells express the M2 isoform (PKM2). Importantly, PKM2 has been shown to play an essential role in mediating the metabolic switch from oxidative phosphorylation of glucose, which is observed in normal cells, to aerobic glycolysis and enhanced lactate production production (Warburg effect) that is observed in cancer cells (Figure 1) [1]. This metabolic reprograming plays a central role in tumor growth and metastasis by providing cancer cells not only with energy but also with the building blocks (nucleotides, amino acids and fatty acids) necessary for proliferation [2-4], and by leading to acidification of the microenvironment [5]. Accordingly, modulation of PKM2 activity is being investigated as a new therapeutic approach for the treatment of cancer. With this in mind, we questioned whether hyperpolarized (HP) ^{13}C MRS of pyruvate could serve as a method to probe the status of PKM2. To this end we compared the fate of hyperpolarized pyruvate in U87 glioblastoma (GBM) cells and in U87 cells in which PKM2 expression was knocked down.

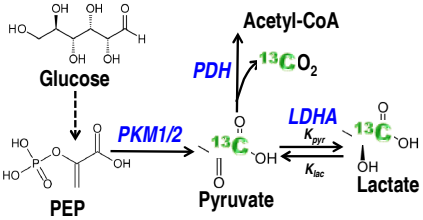

Figure 1 – Schematic of the role of PKM1/2 in metabolism. The hyperpolarized ^{13}C carbon is highlighted in green. PEP: phosphoenolpyruvate; PDH: pyruvate dehydrogenase; LDHA: lactate dehydrogenase A

MATERIAL & METHODS

Cell models To generate PKM2 knockdown cells (U87PKM2_KD), U87 cells were transduced with a viral vector coding for PKM2 shRNA. Control cells (U87_Scr) were generated by transduction with a scrambled shRNA. Cells were cultured as monolayers in high glucose-supplemented DMEM at 37°C in 5% CO_2. Expression levels of PKM2 were assessed by Western Blot analysis. Enzymatic activity of PK was measured using a LDH-coupled assay kit (BioVision).

Live cells studies. To monitor cell metabolism, cells were grown on Biosilon beads and loaded into a 10mm NMR tube and connected to an NMR-compatible perfusion system (bioreactor) as previously described [6]. Briefly, three polyethylene perfusion lines circulated medium to the tube and an additional tube supplied 5% CO_2. A three-way valve allowed for the introduction of hyperpolarized substrate. ^{31}P MRS was performed prior to and after every injection of hyperpolarized pyruvate in order to confirm cell viability.

Hyperpolarization Samples of [1-^{13}C] pyruvic acid were hyperpolarized using the Hypersense DNP polarizer. After approximately 1 hour, pyruvic acid was dissolved in Tris-based buffer and injected into the perfusion system over 20 seconds (final concentration 5mM).

HP ^{13}C MRS ^{13}C spectra were acquired every 3 seconds for 300 seconds using 13° excitation pulses. The intensities of lactate and pyruvate peaks were determined using ACD software and normalized to total hyperpolarized signal and cell number. Lactate-to-pyruvate ratio (Lactate/Pyruvate) was calculated as the ratio of maximum lactate signal to maximum pyruvate signal [6]. A kinetic analysis using Bloch equations modified for chemical exchange was also performed, in order to derive the pseudo-rate constant of lactate formation K_{pyr}, as previously described[6].

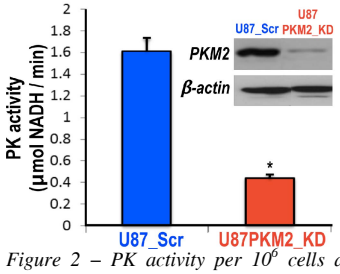

Figure 2 – PK activity per 10^6 cells and PKM2 expression (insert) in U87PKM2_KD and U87_Scr cells

RESULTS & DISCUSSION

Figure 1 illustrates the drop in PKM2 activity observed in U87PKM2_KD (0.47±0.01µmol NADH/min) as compared to U87_Scr control (1.61±0.16µmol NADH/min, *p<0.05*). Western blot analysis confirmed the drop in PKM2 expression (Figure 1, insert). To investigate whether PKM2 status could be monitored by HP ^{13}C MRS, we probed the pyruvate-to-lactate conversion in live cells cultured in a bioreactor system. Figure 3.A presents a stackplot of HP ^{13}C MR spectra acquired post injection of HP pyruvate in perfused U87_Scr cells. The resonances of pyruvate (173ppm), pyruvate hydrate (181ppm), lactate (185ppm), alanine (178.5ppm) and bicarbonate (163ppm) can be observed. After post-processing and quantification, we found that hyperpolarized lactate levels were significantly lower in cells in which PKM2 was knocked down (red) as compared to scrambled controls (Figure 3. B.). Specifically, the lactate-to-pyruvate ratio dropped to 64.6±3.4% of U87_Scr controls in U87PKM2_KD cells (n=3; $p < 0.01$). Kinetic analysis showed that K_{pyr} dropped to 79±7.2% in PKM2 knocked-down cells ($p<0.05$), in line with the drop in lactate-to-pyruvate ratio. No significant difference between the T1 relaxation times could be detected between PKM2_KD and scrambled controls.

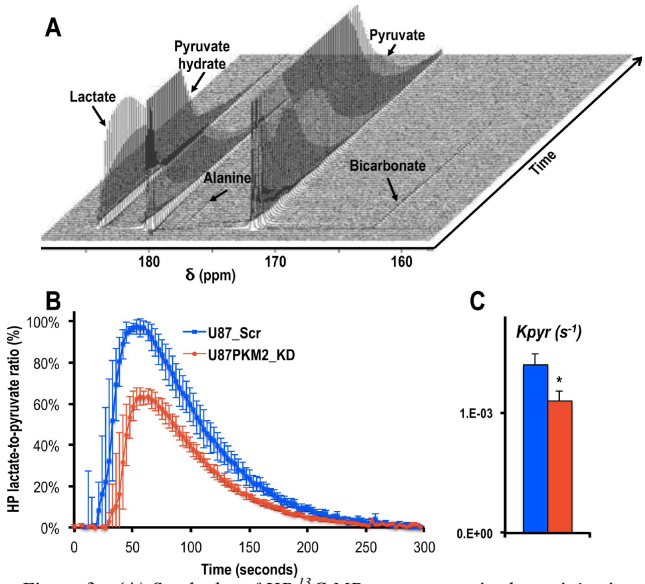

*Figure 3 – **(A)** Stack plot of HP ^{13}C MR spectra acquired post injection of HP pyruvate in perfused U87_Scr cells. **(B)** HP lactate time courses as assessed from HP ^{13}C MR spectra of U87PKM2_KD (red) and U87_Scr (blue) cells. **(C)** Kinetic constant of HP lactate formation Kpyr (s^{-1}) in U87PKM2_KD (red) and U87_Scr (blue) cells.*

As seen in Figure 3.A, bicarbonate could also be detected in our data. However, no significant difference in bicarbonate production could be observed between the two cell lines, possibly due to the relatively low signal-to-noise ratio of this resonance.

Our results are consistent with the role of PKM2 in cancer cell metabolism. Whereas PKM2 converts PEP to pyruvate, it is in a physical complex with multiple enzymes of the glycolytic process, including lactate dehydrogenase A (LDH-A), which converts pyruvate to lactate [7-9]. PKM2 thus indirectly facilitates the conversion of pyruvate to lactate, and PKM2 knockdown would therefore lead to a drop in the conversion of exogenous HP pyruvate into lactate. HP ^{13}C MRS of pyruvate could thus serve to monitor PKM2 expression, and prove useful for evaluation of new PKM2-targeted therapies.

ACKNOWLEDGMENTS This work was supported by Brain Tumor SPORE CA097257, RO1 CA130819, an American Brain Tumor Association fellowship, and center grant P41EB013598.

REFERENCES 1.Christofk HR *et al.,* Nature. 2008;452:230-3. 2. DeBerardinis *et al.,* Cell Metab. 2008;7:11-20. 3. Vander Heiden MG *et al.*, Science. 2009;324:1029-33. 4. Levine AJ *et al.*. Science. 2010;330:1340-4. 5. Gatenby RA *et al.,* Nat Rev Cancer. 2004;4:891-9. 6. Ward *et al.,* Cancer Res. 2010; 70(4):1296-305. 7. Mazurek S *et al.,* Biochem J. 2001;356:247-56. 8. Mazurek S *et al.,* Int J Biochem Cell Biol. 2011;43:969-80. 9. Spoden GA *et al.,* Int J Cancer. 2008;123:312-21.