Noninvasive monitoring of PI3K inhibition: reduced hyperpolarized lactate and PC are independent of genetic background in glioblastoma

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Purpose

The PI3K pathway is activated in 88% of glioblastomas (GBM). Accordingly, this pathway is targeted by many novel therapies currently in clinical trials. Studies have shown that hyperpolarized lactate and phosphocholine (PC) can serve as biomarkers of PI3K inhibition [1,2]. The purpose of this investigation is to study various GBM cell lines, each with different mutations in the PI3K pathway, in order to confirm hyperpolarized lactate and PC as biomarkers of PI3K inhibition in GBM, and to validate these biomarkers by investigating the mechanism for their modulation.

Materials and Methods

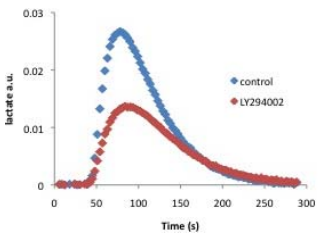
The effect of PI3K inhibition was examined in GBM cell lines GS-2 (PTEN null, EGFR wt), GBM8 (PTEN null, EGFR amplified), and GBM6 (PTEN wt, EGFR mutant), all acquired from Dr. C.D. James (UCSF). Cells were treated for 48 hours with either an inhibitor of the PI3K pathway: 50 μM LY294002 (LY) or 100 nm Everolimus (EVRLS), or with an agent that does not affect PI3K: 100μM temozolamide (TMZ), or 100 μM olomoucine (OLO). For MRS studies, cells were encapsulated in agarose beads and loaded into a perfusion system, modified from previously described (3). MR studies were performed on a 500 MHz INOVA spectrometer (Varian). ^{31}P spectra were acquired using a 30° pulse with 1 sec relaxation. $^{13}C_1$-pyruvate was hyperpolarized using the Hypersense DNP (Oxford Instruments) polarizer as described previously (4). ^{13}C spectra were acquired using a 13° pulse. The intensities of lactate peaks were quantified by integration and normalized to maximum polarization and cell number. To monitor PC synthesis cells were perfused with 1,2-^{13}C choline (Cambridge Isotope Laboratories, MA) over a period of 20 hours. ^{13}C spectra were acquired during that time in 2 hour intervals using a 30° pulse and 3 second relaxation delay. In parallel experiments, the effect of PI3K inhibitor treatment on lactate dehydrogenase (LDH) and HIF-1 levels was determined by standard western blot techniques. Enzyme activity assays were performed to assess the effect of PI3K inhibition on LDH reaction kinetics. MR–based choline kinase activity assays were adapted from previously published work (5).

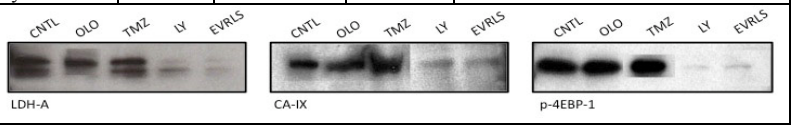
Results

Glucose metabolism

Treatment / target	Cell line	max lactate % control	Vmax % control
LY – PI3K	GS-2	42 ± 12%	48 ± 4%
LY – PI3K	GBM8	53 ± 7%	49 ± 2%
LY – PI3K	GBM6	62 ± 4%	63 ± 9%
Everolimus – PI3K	GS-2	76 ± 5%	64 ± 4%
TMZ DNA damage	GS-2	105 ± 8%	102 ± 9%
OLO – cell cycle arrest	GS-2	124 ± 8%	119 ± 3%

Drop in hyperpolarized lactate in GS-2 LY treated cells

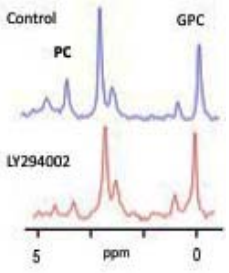




Choline Metabolism

Treatment / target	Cell line	PC % control	De novo PC synthesis	Choline Kinase activity
LY – PI3K	GS-2	34 ± 9%		
LY – PI3K	GBM8	44 ± 6%		
LY – PI3K	GBM6	42 ± 8%		
Everolimus – PI3K	GS-2	62 ±13%		
TMZ – DNA damage	GS-2	111 ± 4%		
OLO – cell cycle arrest	GS-2	102 ± 7%		
control	GS-2		++	0.0016 mM/min
LY – PI3K	GS-2		+	0.0011 mM/min

Drop in PC levels in GS-2 LY treated cells



Our findings are summarized in the table above. The effect of treatment was confirmed in every case by inhibition in cell proliferation and cell cycle arrest (data not shown). Following treatment with PI3K signaling inhibitors a drop in P-4E-BP1 was also confirmed. Hyperpolarized ^{13}C studies illustrated that across genetic backgrounds, hyperpolarized pyruvate to lactate conversion was affected in same way. After treatment with LY294002 or Everolimus, maximum lactate levels dropped in all cell lines. These effects were paralleled by a drop in LDH activity as determined by a drop in V_{max} in cell lysates. Reduced LDH-A expression, as well as a decrease in carbonic anhydrase IX, a reporter of HIF-1, the transcription factor controlling LDH-A expression, were also observed. Additionally, data from ^{31}P spectra indicated that treatment with inhibitors of the PI3K pathway resulted in a drop in PC. To determine the underlying mechanism, *de novo* synthesis of PC was probed using labeled choline. Control cells showed PC labeling, whereas in treated cells labeled PC was below detection level. Additionally, MR-based choline kinase activity assays showed a drop of 33% in LY-treated GS-2 cells. To assess the specificity of our findings, GS-2 cells were treated with olomoucine, a cyclin-dependent kinase inhibitor, and with temozolamide, a DNA damaging agent. As in the case of PI3K inhibition, both treatments resulted in cell cycle arrest but no apoptosis. Importantly, there was no drop observed in either hyperpolarized lactate, LDH activity or PC levels in olomoucine or temozolamide treated cells (n=3, p>0.05).

Discussion

This study indicates that PI3K inhibition upstream of the transcription factor HIF-1 results in a drop in both hyperpolarized lactate and PC, independent of the genetic background of treated cells. Additionally, as HIF-1 is the transcription factor responsible for expression of LDH and choline kinase, which are responsible for catalyzing the pyruvate to lactate conversion and the synthesis of PC respectively, we hypothesize that our observations are mechanistically linked and are associated with a drop in HIF-1 expression as a result of reduced PI3K signaling. These findings suggest a promising application for hyperpolarized lactate and PC as noninvasive metabolic biomarkers of molecular response to PI3K-targeted anticancer drug treatments in patients.

References: [1] Beloueche-Babari et al. Mol Cancer Ther 2006. [2] Ward et al. ISMRM 2008. [3] Ronen and Degani. Magn Reson Med 1989. [4] Kohler et al. Magn Reson Med 2007. [5] Iorio et al. Cancer Res 2005. **Acknowledgements:** Funding: R21 CA120010-01A1, RO1 CA130819, P50 CA097257 ITL-BIO04-10148 in conjunction with GE Healthcare.