In-vivo 13C MRS detects an increase in lactate production associated with PDH down-regulation in genetically engineered mutant IDH1 glioma tumors

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Background: Wild-type isocitrate dehydrogenase (IDH) is the enzyme that catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG) whereas mutant IDH catalyzes the conversion of α-KG into 2-hydroxyglutarate (2-HG). Mutations in IDH1 have been reported in over 70% of low-grade gliomas and secondary glioblastomas (GBM). These mutations are associated with the accumulation of 2-HG within the tumor and are believed to be one of the most early events in the development of low grade gliomas. In a previous study, we used ^{13}C MRS in combination with hyperpolarized (HP) 1-^{13}C pyruvate to monitor the glycolytic pathway and the TCA cycle in U87 cells genetically engineered to express wild-type or mutant IDH1 (U87 IDHwt and U87 IDHmut respectively)[1]. U87 IDHmut cells displayed a significant 159% increase in HP lactate production. The goal of this study was to validate *in-vivo* our previous findings in cells.

Material and Methods: All procedures were performed according UCSF IACUC approval. U87 cells expressing mutant IDH1 and wild-type IDH1 were generated by transduction with a lentiviral vector as described earlier[2]. 6 weeks old athymic nu/nu mice were injected intracranially with 3x10^5 U87 IDHwt or IDHmut cells[3]. MRI studies were performed using a vertical wide bore Agilent 600MHz scanner. Once tumors reached a diameter of 2-3mm, animals were imaged as follows. Axial images were recorded using a spin echo sequence (TE/TR=20/1200ms, FOV=30x30mm, 256x256, ST=1.8mm, NA=2). 1-^{13}C pyruvic acid with 15mM trityl radical OX063 was hyperpolarized using a HyperSense DNP polarizer, followed by dissolution to a 100mM solution in isotonic buffer. 300μl of pyruvate was then injected through an i.v. tail-vein catheter over 12s. ^{13}C MRSI spectra were recorded using 2D CSI dynamic sequence (TE/TR=1.2/60ms, FA=10deg, frequency dimension=128, phase dimension=7x7, SW=2500Hz, FOV=24x24mm). Data were processed using the Sivic software[4]. Lactate peak integrals at each time point were normalized to the maximum pyruvate peak integral. PDH activity was determined using a spectrophotometric assay as previously[5].

Results and discussion: Figure 1 illustrates the location of the HP ^{13}C MRSI grid over the anatomical axial images (top) and the dynamic HP ^{13}C MRSI spectra acquired at 20 seconds post hyperpolarized pyruvate injection (bottom) from the tumor voxels in U87 IDH1wt (left) and U87 IDH1mut (right) animals. Figure 2 illustrates the temporal evolution of the lactate peak and demonstrates that in U87 IDHmut tumors the amount of lactate produced at maximum was 73±7% ($p<0.05$) higher than the amount produced by U87 IDHwt tumors (Fig. 2A). These results confirm our previous study[1] on U87 IDHwt and IDHmut cells (Fig. 2B). The increase in HP lactate detected in our U87 IDH1mut tumor model was associated with a drop in PDH activity in U87 IDHmut cells (Fig. 2C), which likely reflects the metabolic reprogramming of mutant IDH1 tumors.

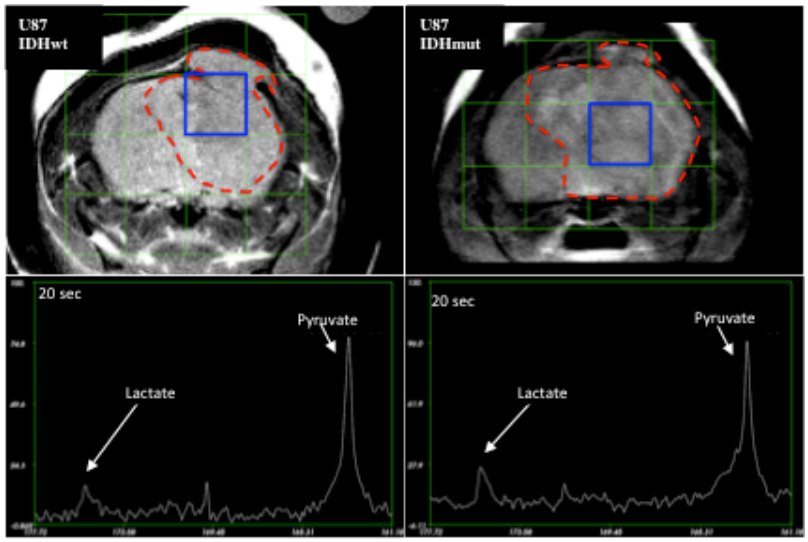

Figure 1: Anatomical axial image and Dynamic ^{13}C MRSI spectra acquired at 20sec after 1-^{13}C pyruvate shot in U87 IDHwt (left column) and IDHmut (right column) tumor.

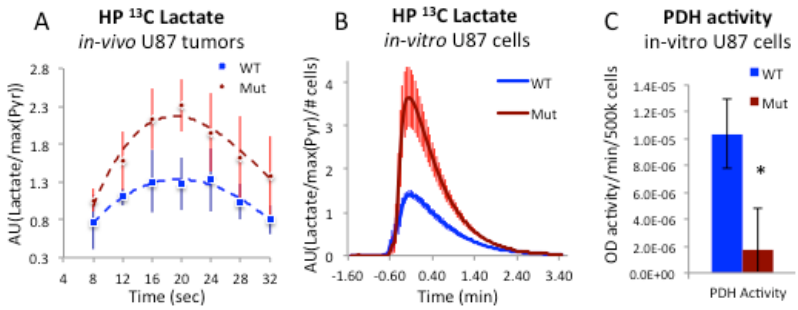

Figure 2: Build up of *de-novo* 13C lactate from 1-^{13} C pyruvate in U87 IDHwt (blue) and IDHmut (red) *in-vivo* tumors (A) and *in-vitro* cells (B). PDH activity of U87 IDHwt (blue) and IDHmut (red) cells.

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References: 1. Izquierdo-Garcia, J.L. *et al.* ISMRM Milan, 0848 (2014). 2. Chaumeil, M. M. *et al. Nat Commun* 4, 2429 (2013). 3. Chaumeil M.M. et al. Neuroimage, 59-1 (2012). 4. Crane J.C. *et al.* Inter. J. Biomed. Imag, 169526(2013). 5. Izquierdo-Garcia *et al.* PLoSOne 9(9) e108289(2014)