**In vivo detection of PI3K pathway inhibition by hyperpolarized $^{13}$C MRSI at 14 Tesla**


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**INTRODUCTION**
Glioblastoma (GBM) is the most common and lethal primary malignant brain tumor in humans [1]. Despite surgical resection, chemotherapy and radiotherapy treatments, the median survival for GBM patients is ~1 year [2]. One of the new promising treatment approaches targets the phosphatidylinositol-3-kinase (PI3K) signaling pathway, which plays a crucial role in cell growth, proliferation and survival and is frequently activated in human cancers [3]. In particular, the anticancer drug Everolimus (RAD001), an inhibitor of the PI3K downstream effector mTOR, is currently in phase II clinical trials. However, the assessment of response to PI3K/mTOR inhibitors using traditional imaging methods remains a challenge, as drug action is often associated with tumor stasis rather than shrinkage. Using hyperpolarized (HP) $^{13}$C magnetic resonance spectroscopy (MRS), the effect of PI3K/mTOR inhibition was previously investigated by monitoring HP $^{[1-13]}$-lactate levels produced from HP $^{[1-13]}$-pyruvate in cells [4,5]. These studies showed a drop in HP lactate associated with a drop in lactate dehydrogenase (LDH) expression and activity resulting from a drop in the hypoxia inducible factor (HIF-1), which is controlled by PI3K/mTOR signaling. Based on these findings, we designed a study aimed at detecting PI3K/mTOR inhibition by Everolimus in tumors in vivo using HP $^{13}$C MRS at 14 Tesla.

**MATERIAL & METHODS**

**Tumor-bearing animals** 4 weeks-old athymic mice (Nu/Nu, Simonsen, Gilroy, CA) were included in the study. For tumor implantation, animals were anesthetized using ketamine/xylazine (100/20 mg/kg respectively) and a suspension of GS2 cells (~1x10^7) was injected in the left flank. When the tumor reached a diameter of ~6mm, treated animals received a daily intraperitoneal injection of Everolimus (10mg.kg^-1.day^-1) in DMSO, v=20μL, Molcan Corporation, Canada) while control animals received the same volume of DMSO. During treatment, tumor size was monitored by caliper measurement. All experimental procedures were approved by the UCSF Institutional Animal Care and Use Committee.

**MR system & Experimental set-up** Experiments were performed on a 600 MHz wide bore vertical NMR system (Ø I=55 mm, 100 G.cm^-1 gradients, Varian Inc, Palo Alto, CA). MR imaging was performed using a Varian millipede $^1$H coil ($\phi$=40mm, 5cm length). A custom-built $^1$H surface coil ($\phi$=20mm) was used for hyperpolarized studies. Mice were anesthetized using isoflurane (3% in $O_2$, 1.5 L.min^-1) and a 27G catheter was secured in the tail vein of the animal. The tumor region was placed in the center of the $^1$H coil, and the animal was positioned in the magnet using a custom built cradle. A glass tube containing $^{13}$C-enriched urea ($c=10M$, $O_2=4mm$) placed at the center of the surface coil was used for position and chemical shift reference. Temperature and respiration were monitored throughout the experiment.

**MR acquisitions** Anatomical imaging was first performed to assess the positioning of the tumor and of the urea sample (2D Spin Echo (SE), coronal, TE/TR=20/2000ms, FOV=32x32mm, matrix 256x256, slice thickness=0.5mm, gap=0.5mm, at=8min32s, NT=2). $[1-13]$-pyruvatic acid (Isotech; Champaign, IL) containing 15 mM of the trityl radical OX063 (Oxford Instruments; Abingdon, UK) was hyperpolarized using the HyperSense DNP polarizer (Oxford Instruments as described previously [6, 7]. After 1 hr, polarized pyruvic acid was rapidly dissolved in isotonic buffer (40mM Tris, 100mM NaOH, 0.11mg/L NaEDTA) to obtain a 100mM solution. Within less than 10s, 300μl of this solution was injected through the iv catheter over 12s. $^{13}$C 2D-MRSI was acquired 37s after injection, the time point when, based on non-localized $^{13}$C dynamic data, the hyperpolarized $^{13}$C lactate reached a maximum as previously described [8]. The $^{13}$C 2D-MRSI parameters were as follows: TE/TR=0.195/125ms, frequency dimension=512, phase dimension=8x8, SW 5000Hz, FOV=32x32mm, at=8s). A rectangular pulse (pw=100us) equivalent to 20$Ω$ FA at 5mm from the coil was used for excitation.

**Results & discussion** Figure 1 presents data obtained from one animal. The left column corresponds to data acquired before treatment; the right column to data acquired 2 days post Everolimus treatment. Coronal SE reference images (1A) acquired 7mm from the surface coil allow assessment of the location and size of the tumor. The tumor voxels were considered as tumor voxels and the lactate-to-pyruvate ratios from these voxels were averaged.

**Fig 1 - Effect of Everolimus treatment on GS-2 tumor xenografs (A) Coronal SE image overlaid with tumors voxel (B) Corresponding 2D-MRSI spectra and (C) lactate-to-pyruvate ratio maps.**

**REFERENCES**

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