Measuring Tumor Metabolism in Diffuse Intrinsic Pontine Gliomas (DIPG) Using Hyperpolarized Carbon-13 MR Spectroscopic Imaging

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Introduction: Diffuse intrinsic pontine gliomas (DIPGs) are malignant tumors that are one of the most difficult pediatric cancers to treat. The methods for assessing treatment response and tumor progression are based on radiographic response but conventional MRI is not sufficient for predicting clinical outcome. Delineation of tumor boundaries is challenging because the extent of contrast enhancement and T2 hyperintensity are variable among patients and non-specific. A recent first-in-human study using hyperpolarized ^{13}C MR metabolic imaging showed the safety and feasibility of this technology for evaluating hyperpolarized pyruvate metabolism in humans[1]. The purpose of this study was to demonstrate the feasibility of using hyperpolarized ^{13}C metabolic imaging with [1-^{13}C]pyruvate as a non-invasive imaging tool to evaluate *in vivo* metabolism in orthotopic brainstem xenografts injected with human DIPG cells.

Methods: Primary human DIPG cells were implanted into the brainstem of athymic rats (n=4). All experiments were performed using a GE clinical 3T scanner with a custom-designed ^{1}H/^{13}C coil. We pre-polarized 35µL of [1-^{13}C]pyruvate (with 1.5 mM gadolinium) using a HyperSense® DNP polarizer (Oxford Instruments, Abingdon, UK)[2]. Compressed sensing [13]C 3D magnetic resonance spectroscopic imaging (MRSI) data were acquired using a double spin echo sequence (TE/TR=140/215 ms) with centric k-space encoding, a variable flip angle scheme and flyback echo-planar readout on the z-axis at 20 s from the start of the injection of approximately 2.5 mL hyperpolarized [1-^{13}C]pyruvate through the tail vein[3]. The injection started 10 s after dissolution and lasted 10 s. The final dissolved solution had a concentration of 100 mM pyruvate and pH of 7.5. The lactate and pyruvate signals in the brainstem were normalized with respect to the relative signals in normal appearing brain in the supratentorial region. T2-weighted fast spin echo (FSE) images (TE/TR=60/4000 ms) and Gadolinium (Gd)-enhanced (0.2 mmol/kg Gd-DTPA) T1-weighted spin-echo (SE) images (TE/TR=10/700 ms) were acquired in an axial plane. The brains of rats were resected and stained with hematoxylin and eosin (H&E) for histological analysis.

Results: Figure 1 shows an example of rats injected with DIPG cells. The axial T2 FSE images exhibited T2 hyperintensity (Fig 1a), while T1 post-Gd images displayed no contrast enhancement (Fig 1b). Figure 1c shows a sagittal image showing 5.4 mm slice around the brainstem where the representative ^{13}C spectra in Figure 1d were acquired. The hyperpolarized ^{13}C MRSI data exhibited an abnormal ^{13}C metabolism in the lesions (Fig 1d). The mean normalized lactate, normalized pyruvate and the ratio of lactate to pyruvate in the T2 hyperintense voxels from four rats were 3.1 ± 1.7, 1.5 ± 0.4 and 0.47 ± 0.2 (mean ± standard deviation), respectively. The lactate signal in theses areas were highly elevated compared to the lactate signal in the normal appearing brain tissue from the supratentorial region (Fig 1e). The normalized lactate map in Figure 1e shows the differentiation of lactate signal between tumor core, tissue in the periphery of tumor and tissue in the contra-lateral hemisphere. The corresponding H&E stained slides showed viable tumor that recapitulated the histopathology of a subset of high-grade pediatric astrocytomas (Fig 1f).

Conclusions: We have demonstrated the feasibility of using hyperpolarized ^{13}C metabolic imaging for assessing *in vivo* metabolism in an orthotopic human xenograft model of DIPG in rat brain. Highly elevated lactate signal was observed in non-contrast enhancing brainstem lesions relative to the normal appearing brain from the supratentorial region. The results from this study suggest that this technique may provide a unique non-invasive imaging tool that is able to differentiate between tissue pathologies and aid in management of DIPG patients.

References: [1]Nelson et al., Sci Transl Med. 2013. [2]Ardenkjaer-Larsen et al., *Proc Natl Acad Sci USA*. 2003. [3]Park et al., *Magn Reson Med*. 2012.
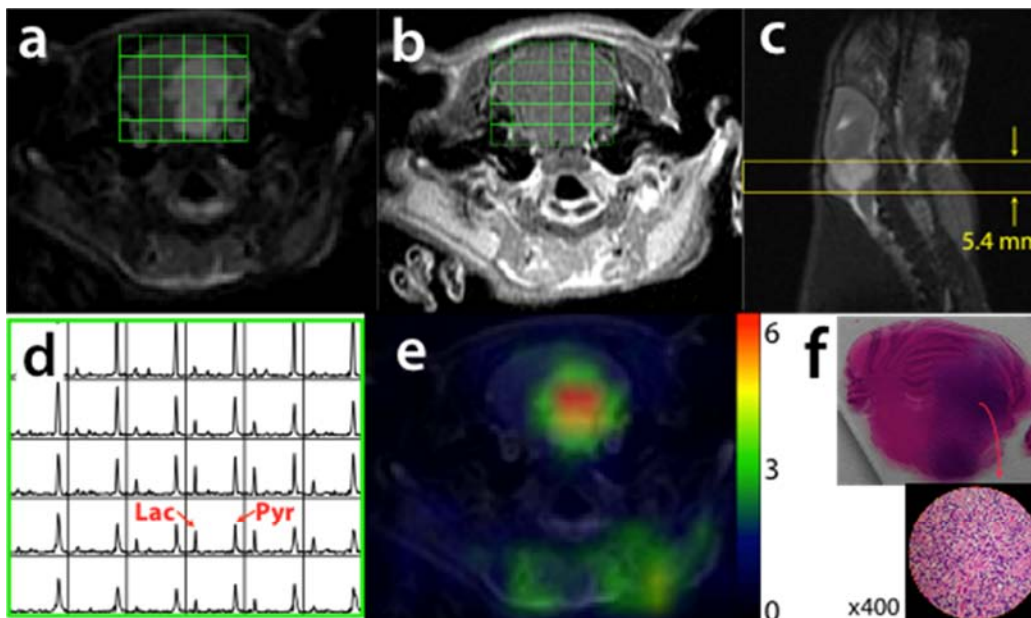
Figure 1. An example of rats with DIPG: a) an axial T2-weighted image showing hyperintense lesion, b) the corresponding post-Gd image demonstrating no contrast enhancement, c) a sagittal image showing a 5.4 mm slice where the ^{13}C MRSI data were acquired, d) ^{13}C spectra showing elevated lactate signal in T2 hyperintense region, e) the map of lactate signal normalized by lactate signal in normal appearing brain tissue in supratentorial region, f) the corresponding H&E slide showing a histological characteristics of high grade pediatric astrocytomas