Metabolic Characterization of Intracranial Lymphoma at 14T using Frequency-Selective 3D Echo-planar ^{13}C Imaging

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INTRODUCTION: Determining the extent of intracranial tumors is critical for surgical resection and focused radiotherapy, but is often difficult by traditional ^{1}H imaging techniques. This problem has been addressed by ^{1}H MRS, positron emission tomography (PET), and most recently hyperpolarized (HP) ^{13}C MR spectroscopy studies in preclinical models [1]. However, HP ^{13}C studies have generally been limited by low spatial resolution, with significant volume averaging limiting interpretation. Here we report application of fast 3D echo-planar imaging (EPI) ^{13}C imaging methods to the murine brain at 14T, in a primary central nervous system lymphoma (PCNSL) xenograft model. This model was chosen since (1) PCNSL can be challenging to diagnose and manage (2) elevated lactate dehydrogenase (LDH) is a lymphoma biomarker suggesting the utility of [1-^{13}C] pyruvate and (3) PCNSL is highly infiltrative, requiring methods capable of detecting subtle metabolic differences. Recently, we employed gas chromatography- mass-spectroscopy (GC-MS) to profile a broad spectrum of metabolites in the CSF of human patients. Metabolite concentrations in CSF from 15 subjects with active PCNSL were compared to 15 controls without brain tumors. Despite similar tumor burdens, the CSF concentrations of lactate were higher in patients with refractory lymphoma compared to chemotherapy-sensitive tumors (**Figure 1**). Our hypothesis is that this elevated CSF lactate correlates directly with the PCNSL microenvironment, making disease progression amenable to metabolic evaluation with HP [1-^{13}C] pyruvate. Here we report preliminary data in a PCNSL xenograft model validating multi-parametric ^{1}H and ^{13}C HP imaging at 14T with significantly improved spatial resolution in the murine brain.

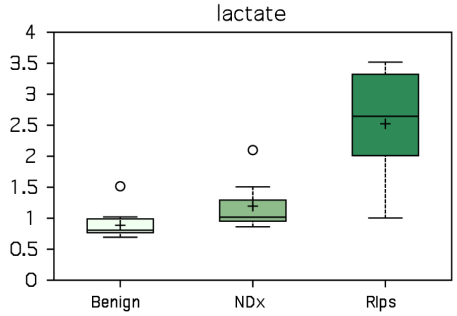

Figure 1. GC-MS data obtained from the CSF of controls (N=15), newly diagnosed cases (N=9) and relapsed/ refractory cases (N=6) indicating levels of relative expression (y-axis).

METHODS: ***Murine lymphoma xenografts:*** Implantation of a Burkitt's cell line (Raji) into the brains RAG-deficient mice was performed, with tumor progression monitored by bioluminescence. MR imaging was performed at 10-12 days and the mice sacrificed immediately thereafter. ***Polarization and dissolution of [1-^{13}C] pyruvate:*** Polarization of [1-^{13}C] pyruvate was performed by the typical method [2] on the Oxford Hypersense Polarizer (Oxford, UK). ***MR imaging methods:*** *In vivo* MR studies were preformed on a 14T, 600WB micro-imaging spectrometer equipped with 100G/cm gradients (Varian Instruments). Multi-slice T_2-weighted anatomic images were acquired using a respiratory-gated spin-echo sequence with a T_E=20ms, T_R=1200ms, fat saturation, FOV 40 x 40 mm, 256 x 256 points (RO x PE). Diffusion-weighted images were acquired using a spin-echo based sequence with similar parameters, 4 B-values (102, 305, 406, and 508) and apparent diffusion coefficient maps were calculated. For ^{13}C pyruvate studies, an echo planar imaging based pulse sequence has been constructed using frequency-specific pulses (*f* = pyruvate, lactate or urea and *n, N*=12) to generate a 3D image for each metabolite with an acquisition time of approximately 180ms (**Figure 2**). Postcontrast T_1-weighted imaging was performed using a standard gradient echo (GE) sequence following administration of intravenous Gd-chelate (Magnevist) at 4 mmol/kg. ***Histologic analysis*****:** After imaging, histopathologic analysis including standard H&E staining was performed on tumors. ***Data processing and analysis*****:** 14T in *vivo* MRI data was processed using custom software written in Matlab 2009b (MathWorks, MA, USA) and IDL 8 (ITT Visual Information Solutions, CO, USA). Data were zerofilled and transformed to a final resolution of 1.25mm isotropic (0.002cc).

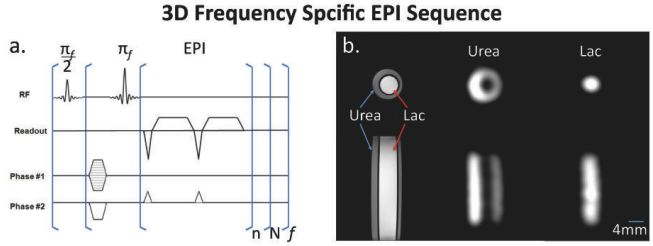

Figure 2. (a) Diagram of 3D frequency specific imaging pulse sequence utilizing both frequency specific 90° and 180° pulses. (b) Phantom images demonstrating the application of the 3D frequency specific imaging sequence with a corresponding reference ^{1}H gradient echo image. The inner tube contains 4M [1-^{13}C] lactate, while the outer tube contains 4M ^{13}C Urea. f = frequency, N, n = 12 or 16, resolution of 1.25mm x 1.25mm x 1.25mm, Lac = lactate.

RESULTS: *In vivo* studies in Raji mice were performed at 14T, with correlative histopathology. The results are shown in **Figure 3**, demonstrating significant differences between lymphoma and normal brain using both conventional ^{1}H imaging and HP ^{13}C pyruvate. In these studies, T_2-weighted imaging demonstrated relatively subtle areas of T_2 prolongation, significantly reduced ADC values, and central foci of avid post-gadolinium enhancement in tumor, corresponding to areas of infiltrating hypercellularity observed in the pathologic specimen. HP [1-^{13}C] pyruvate imaging demonstrated striking elevation in the metabolite [1-^{13}C] lactate in tumor, with the abnormal signal extending beyond the gross abnormality identified histologically.

DISCUSSION: Elevated serum lactate dehydrogenase (LDH) is a hallmark of lymphoma, and correlates with disease relapse/ recurrence in several lymphoma subtypes [3]. As for other cancers, enhanced expression of LDH (with a concomitant rise in lactate) reflects a primitive phenotype shifted away from oxidative phosphorylation and towards glycolyis, consistent with the Warburg hypothesis [4]. This phenotype not only reflects the elevated energy demands of tumor, but also relates to tumor progression, with acidification of the tumor microenvironment promoting local invasion [5]. Our hypothesis that changes in CSF lactate seen in PCNSL patients relate directly to tumor adaptation and disease aggressiveness. Preliminary data obtained in a PCNSL xenograft model employing multi-parametric ^{1}H and ^{13}C methods at 14T suggest that this may be the ideal platform to investigate fundamental metabolic changes in brain tumors.

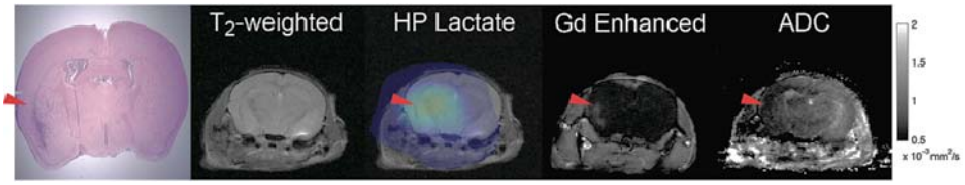

Figure 3. Typical MR data obtained for an implanted Burkitt's lymphoma (Raji) mouse at 14T. Corresponding to the region of infiltrating tumor identified on H&E staining, signal abnormality was seen on T_2 FSE, hyperpolarized ^{13}C lactate imaging, T_1 GE post-gadolinium, and apparent diffusion coefficient (ADC) mapping.

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