MR Spectroscopic Imaging of Lactate in Dedifferentiated Liposarcoma Models

A. Rizwan[1,2], X. Ni[1], R. O'Connor[3], S. Singer[3,4], J. Koutcher[1,5], and K. L. Zakian[1,5]

[1]Medical Physics, Memorial Sloan-Kettering Cancer Center, New York, NY, United States, [2]Weill Cornell Medical College, New York, NY, United States, [3]Sarcoma Biology Laboratory, Sarcoma Disease Management Program and Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY, United States, [4]Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY, United States, [5]Radiology, Memorial Sloan-Kettering Cancer Center, New York, NY, United States

Introduction: Liposarcoma comprises 20% of all soft tissue sarcomas (STS). Dedifferentiated liposarcoma (DDLS) is an aggressive STS subtype with 5-year disease-specific survival of 44% (1). Not only is there a need for more effective treatment for this potentially deadly tumor, but additional prognostic information would be useful in identifying patients needing more aggressive treatment. Lactate has been shown to be a marker of poor prognosis in several solid tumor types (2-5). The goal of the current study was to assess the glycolytic phenotype in two models of DDLS using lactate-edited MR spectroscopic imaging. Two different DDLS xenograft types derived from patients at our institution were studied.

Methods: Animal preparation: Seven BWH and 4 DDLS8817 tumors were grown in the flanks of SCID mice and monitored until the volume was approximately 150mm^3. MRI/MRSI was performed serially at 3 different tumor volumes in each mouse. Tumor volume (V) was calculated as V= (π/6)* L * W * D. **MR Experiment:** Mice were anesthetized with Isoflurane during the MR experiments on a Bruker 7-T Bruker Biospec Spectrometer. Two home built 2-turn solenoid coils with diameters of 10 mm and 16 mm were used. T2-weighted sagittal MR images were collected using the RARE sequence (slice thickness = 1mm, FOV =24 mm, TR = 2217 ms, TE = 40 ms, matrix size 512 X 256, number of average = 8). Lactate detection was performed using the Selective Multiple Quantum Coherence (Sel-MQC) editing sequence (6,7). Lactate spectra from both the whole tumor and 5-mm thick center slice were acquired with TR = 2 sec, number of excitations =16, 1024 data points, and spectral width of 5952Hz. Two dimensional chemical shift imaging (2D CSI) of the 5-mm thick slice was obtained. The parameters of the 2D CSI are: matrix size 16 X 16, FOV = 24 mm (1.5 x 1.5 mm in plane resolution, 11.25 mm^3 voxel volume) and acquisition time of 70 minutes. **Histology:** Selected animals were injected with Hoechst 33342 (40 mg/kg) at 5 minutes pre sacrifice to assess tumor perfusion. Tumors were excised, embedded in cutting medium (OCT 4583, Sakura Finetek), snap-frozen and stored at −80°C. 10μm thick frozen sections were obtained using a cryostat microtome (Microm International GmbH). Sections were fixed in 4% paraformaldehyde then blocked in Superblock-PBS (Pierce, USA) followed by hematoxylin/eosin staining.

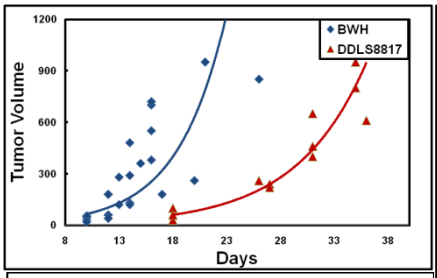

Fig 1: Growth Curves: BWH tumor doubling time = 2.2d, DDLS8817 doubling time 4.2 d.

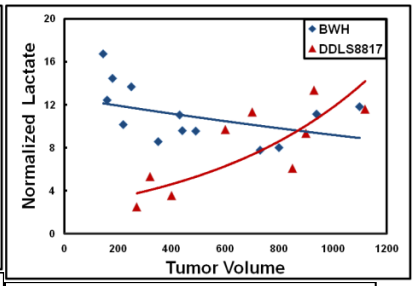

Fig 2: Normalized serial lactate levels in 5mm slices of tumor sections.

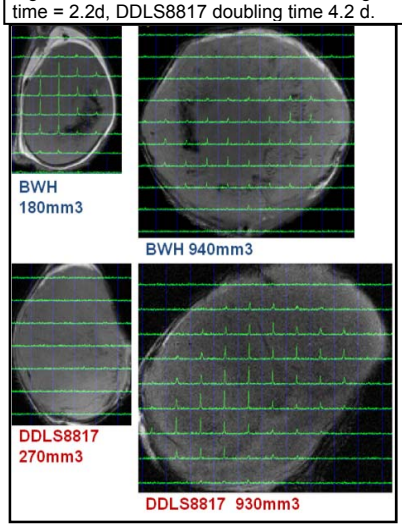

Fig 3:SEL-MQC edited lactate CSI in BWH and DDLS8817 at small and large volumes

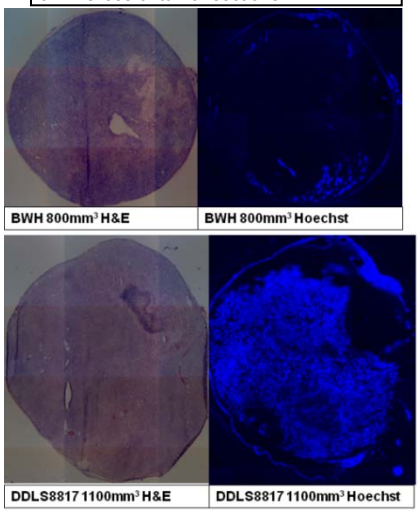

Fig 4: BWH large tumor H&E(A) and perfusion (B). DDLS8817 large tumor H&E(C) and perfusion(D).

Data Analysis: For whole-slice spectra, the lactate peak area was normalized by the slice volume and compared to the lactate content of a phantom section of known volume and concentration. **Results:** The BWH tumors grew more rapidly than the DDLS8817 (Fig-1). The BWH tumors exhibited high lactate concentrations at small tumor volumes (150-250 mm^3) while the DDLS8817 had very low lactate at low tumor volumes (250-400mm^3) (Figs. 2 and 3). As BWH tumors increased in size there was an associated decrease in lactate levels. In contrast, in DDLS8817 the lactate levels increased exponentially with tumor growth. Histology of large tumors showed that BWH have necrotic regions (Fig4A) and less Hoechst uptake (Fig4B) compared to the high Hoescht uptake and minimal necrosis seen in DDLS8817) tumors (Fig4D) (Fig4C).

Discussion: Gene expression studies have shown upregulation in genes and proteins involved in cell cycle and proliferation in DDLS cells compared to normal adipocytes (8,9). In addition, genes coding for pyruvate carboxylase and phosphoenolpyruvate carboxykinase 1 (pck1) which are involved in pyruvate entry into gluconeogenesis are downregulated in DDLS (10) and a recent study has shown that pck1 gene expression is independently associated with recurrence free survival in liposarcoma (HR=.72) (unpublished). Furthermore, growth rates of locally recurrent DDLS in humans have been associated with survival (11). Based on these studies non-invasive measures of tumor growth rate and lactate production would be of considerable prognostic value for patients with DDLS. Our results show that there is heterogeneity in lactate production in xenografts derived from different human DDLS tumors and that lactate levels are higher in the rapidly growing BWH tumors at small volumes while lactate levels are lower in the slower growing DDLS8817 line at small volumes. As these are whole-slice data, the reduced lactate in the larger BWH tumors could be Due to necrotic regions. This appears to be supported by histology. Correlation of the spatial distribution of lactate in the CSI maps with histology is ongoing. The high level of lactate in the smaller BWH tumors suggests a more glycolytic and aggressive phenotype. This work illustrates the value of MRS for noninvasively assessing tumor metabolism and providing a potential clinically translatable prognostic marker. Assessment of the relative expression levels of genes/proteins related to glycolytic metabolism such as LDH-A, pyruvate carboxylase and phosphoenolpyruvate carboxykinase is planned to determine if lactate MRS reflects genotypic differences in the two tumor lines.

References 1. Dalal KM, et. al. Ann Surg 2006;244(3):381-391. 2. Brizel DM, et.al. Int J Radiat Oncol Biol Phys 2001;51(2):349-353. 3. Walenta S, et. al. J Cancer Res Clin Oncol 2003;129(6):321-326. 4. Walenta S, et. al. Am J Pathol 1997;150(2):409-415. 5. Walenta S, et. al. Cancer Res 2000;60(4):916-921. 6. He Q, et. al. J Magn Reson B 1995;106(3):203-211. 7. Murugananandham M, et. al. Magn Reson Med 2004;52(4):902-906. 8. Brill E, et. al. Cancer Res 2010;70(17):6891-6901. 9. Singer S, et. al. Cancer Res 2007;67(14):6626-6636. 10. Angeles CV, et. al. J Clin Oncol (Meeting Abstracts) 2010;28(15_suppl):10005-. 11. Park JO, et.al. Ann Surg 2009;250(6):977-982.