Comparison of Hyperpolarized [1-^{13}C] Dehydroascorbate-MR and FDG-PET in a Transgenic Prostate Cancer Model

Kayvan R. Keshari[1], Victor Sai[1], Kristen Scott[1], John Kurhanewicz[1], Henry F. VanBrocklin[1], and David M. Wilson[1]

[1]*Radiology and Biomedical Imaging, University of California, San Francisco (UCSF), San Francisco, CA, United States*

INTRODUCTION: Hyperpolarized (HP) ^{13}C MR studies of the transgenic model of prostate cancer (TRAMP) model have demonstrated that hyperpolarized (HP) [1-^{13}C] pyruvate can detect both local and metastatic prostate cancer, provide an assessment of pathologic grade [1], and determine early response to therapy [2]. This finding has lead to the recent clinical translation of HP [1-^{13}C] pyruvate in the first human trial of HP MR in prostate cancer patients [3]. Recently, we have developed a new probe for *in vivo* imaging of reduction and oxidation (redox), HP [1-^{13}C] dehydroascorbate (DHA) [4]. HP DHA is rapidly taken into the cell via glucose (GLUT) transporters and then reduced to ascorbate (VitC). This conversion takes place rapidly in the brain, liver, kidneys, and TRAMP tumor. These tissues are known to harbor large concentrations of glutathione (GSH), primarily responsible for reduction of DHA in vivo in coupled redox reactions with NADPH, mediated by several intracellular enzymes including glutaredoxin [5]. Our hypothesis is that the observed reduction of HP [1-^{13}C] DHA *in vivo* relates to both cellular GSH concentration, as well as increased glucose uptake. The gold standard for metabolic imaging, 2-^{18}F-2-deoxy-D-glucose (FDG) used in positron emission tomography (PET) imaging, takes advantage of increased glucose uptake to generate contrast between tumor and normal tissues. Since [1-^{13}C] DHA has an essentially identical transport (and charge trapping) mechanism, we speculated that it might have considerable overlap with FDG in tumor models. The goal of this study was to compare the increased HP [1-^{13}C] VitC signals observed by MRSI to accumulation of FDG-PET in a cohort of TRAMP mice.

METHODS: ***Hyperpolarization and dissolution of [1-^{13}C] DHA:*** [1-^{13}C] DHA dimer was polarized and dissolved as described previously [6]. ***3T MR Studies:*** 3D MRSI studies were performed using a 3T MRI scanner (GE Healthcare, Waukesha, WI) equipped with the MNS (multinuclear spectroscopy) hardware package and a dual-tuned mouse coil as previously described. ***Data Processing and Analysis:*** *In vivo* MRSI data was processed using custom software written in IDL 8 (ITT Visual Information Solutions, CO, USA) and Matlab 2009b (MathWorks, MA, USA). Average metabolite ratios (VitC/ [VitC + DHA]) were calculated for voxels corresponding to both tumor and surrounding benign tissues in TRAMP mice (N=4). ***FDG-PET:*** PET scans were performed on a small animal PET/CT scanner (Inveon, Siemens Healthcare, Malvern, PA). Mice were fasted overnight, anesthetized, and injected intravenously with 150-170uCi FDG in 0.1-0.2mL saline. PET images were acquired 50 minutes post injection in one 600 second frame. CT images were acquired in 120 projections of continuous rotation to cover 220° with x-ray tube operating at 80 kVp, 0.5 mA, and 175 ms exposure time. The matrix size of the reconstructed CT images was 512×512×662 with an isotropic voxel size of 0.191×0.191×0.191 mm^3. PET images were reconstructed using a manufacturer-provided ordered subsets expectation maximization (OS-EM) algorithm resulting in a 128×128×159 matrices with a voxel size of 0.776×0.776×0.796 mm^3. ROIs were drawn in both the tumors and surrounding tissue to calculate relative tumor signals.

RESULTS AND DISCUSSION: Relative to benign prostate tissue, TRAMP tumors (N=4) demonstrated increased cellularity (100% poorly differentiated), proliferative rates (88±7% KI-67 positive cells) and hypoxia (19.4±6% PIM positive cells) (**Figure 1a**). These cells tend to be highly glucose avid and reduced. High levels of HP VitC are observed in these TRAMP tumors post-injection of HP DHA (0.23±0.03) significantly higher than that of surrounding tissues (0.05±0.02, P=0.005, **Figure 1c**) and benign prostate (0.06±0.03, P=0.001). When imaged using PET-FDG these mice also demonstrated increased ^{18}F signal in the regions of tumor relative to surrounding regions (6.8±3.6, **Figure 1d**). ^{18}FDG signals in the normal prostate are difficult to quantify because of its proximity to the bladder. ^{18}FDG signals in the TRAMP tumors appeared fairly homogenous (**Figure 1b**). Total HP ^{13}C in tumors was elevated relative to surrounding tissue (2.1±0.4), but this was significantly less than the tumor HP VitC/ [VitC + DHA] relative to surrounding tissue (7.0±2.3). Interestingly, regions of differing VitC/[VitC + DHA] were seen in the tumors that may represent different concentrations of reducing agents. Future studies will be needed to elucidate these regional differences in reducing capacity.

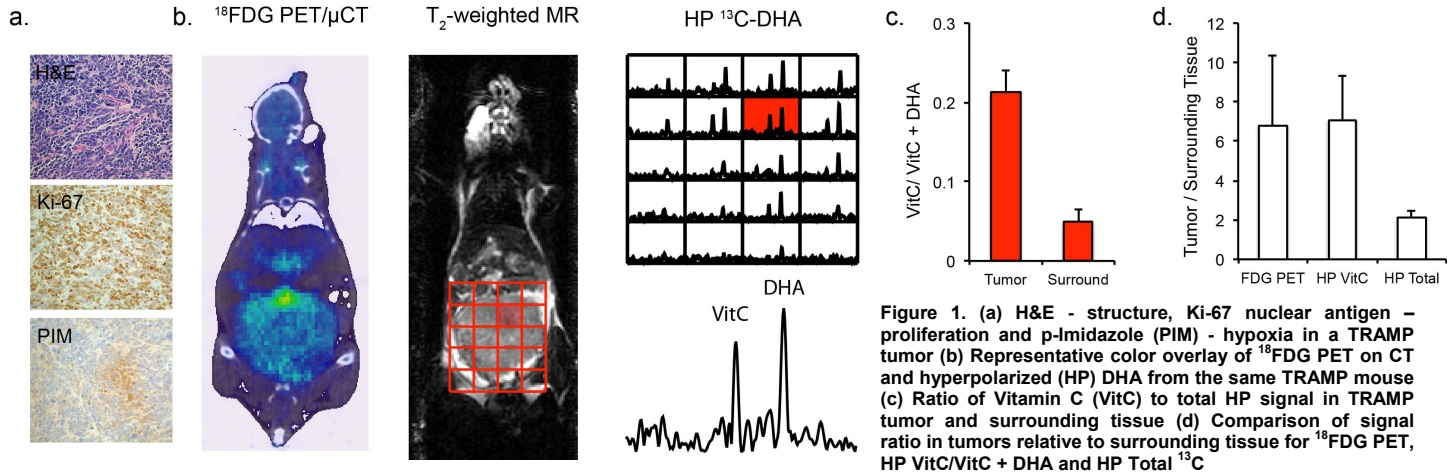

Figure 1. (a) H&E - structure, Ki-67 nuclear antigen – proliferation and p-Imidazole (PIM) - hypoxia in a TRAMP tumor (b) Representative color overlay of ^{18}FDG PET on CT and hyperpolarized (HP) DHA from the same TRAMP mouse (c) Ratio of Vitamin C (VitC) to total HP signal in TRAMP tumor and surrounding tissue (d) Comparison of signal ratio in tumors relative to surrounding tissue for ^{18}FDG PET, HP VitC/VitC + DHA and HP Total ^{13}C

CONCLUSIONS: ^{18}FDG-PET plays an important role in the standard of care for cancer patients, but has limitations in prostate due to bladder contamination effects and difficulty distinguishing perfusion from metabolism. HP DHA provides a means to look at perfusion (the sum of VitC and DHA) as well as *in vivo* conversion (ratio of VitC/[VitC+DHA]). In the TRAMP model, increased reduction of HP [1-^{13}C] DHA was observed in tumor, correlating with both elevated glucose uptake (as seen in ^{18}F FDG studies), and high concentrations of GSH [7]. Since alterations in redox have been implicated in tumor aggressiveness and resistance to therapy, HP [1-^{13}C] DHA could be used to predict and monitor treatment outcomes.

REFERENCES: [1] Albers MJ *et al*. 2008. *Cancer Res* 68(20): 8607-15. [2] Chen A *et al*. 2008. *ISMRM*. [3] Kurhanewicz J *et al*. 2011. Unpublished results (UCSF ^{13}C clinical trial). [4] Keshari K *et al*. 2011. PNAS (Epub ahead of print 10/11). [5] Linster CL *et al*. 2007. *FEBS* 274: 1-22. [6] Ardenkjaer-Larsen JH *et al*. 2003. *PNAS* 100(18): 10158-10163. [7] Levin YS *et al*. 2009. *MRM* 62(5): 1091-1098.

ACKNOWLEDGEMENTS: Grant sponsors NIH P41 EB013598, RSNA RSD1014.