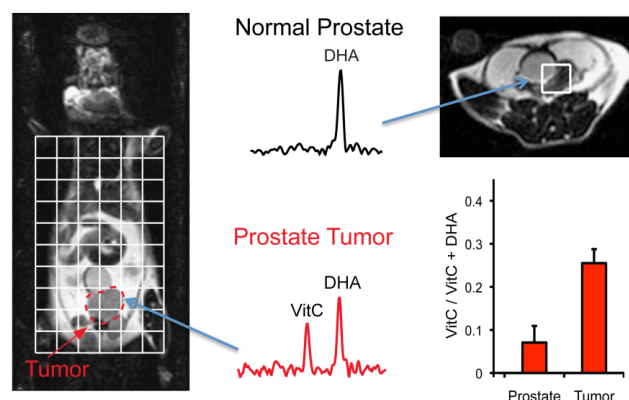


# Molecular Correlates to *in vivo* Hyperpolarized [1-<sup>13</sup>C] Dehydroascorbate Reduction

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**INTRODUCTION:** Hyperpolarized (HP) [1-<sup>13</sup>C] dehydroascorbate (DHA) is a recently developed probe that has demonstrated potential for *in vivo* imaging of reduction and oxidation (redox), which is highly dysregulated in cancer cells. <sup>13</sup>C MRSI studies with HP DHA in a transgenic model of prostate cancer (TRAMP) model have shown rapid uptake of HP DHA into the cell via glucose (GLUT) transporters with subsequent reduction to ascorbate (vitamin C), from which the redox state of the tissue can be assessed [1]. This redox state is also known to correlate with the concentration of glutathione (GSH) and non-protein thiols, which in conjunction with several intracellular enzymes including thioredoxin reductase, mediate reduction of DHA to vitamin C [2,3]. High glutathione levels have been associated with cancer cell resistance to therapy [4], and clinical studies have found higher quantities of glutathione in tumors compared to normal tissue [3]. Similarly, thioredoxin reductase has been shown to be elevated in certain human cancer cell lines and has been correlated with increased aggressiveness [5]. We hypothesize that the observed reduction of HP [1-<sup>13</sup>C] DHA *in vivo* correlates with (1) uptake via GLUT-type transporters, (2) the intracellular GSH concentration, and (3) intracellular enzymes involved with the reduction of DHA. The purpose of this study is thus to correlate the high HP [VitC] signals observed by MRSI in the tumors of TRAMP mice with transporter expression, intracellular glutathione concentration, and enzymes involved in regulating redox.



**Figure 1.** Coronal T<sub>2</sub>-weighted image of TRAMP and axial T<sub>2</sub>-weighted image of a normal mouse prostate with corresponding hyperpolarized <sup>13</sup>C MR spectra post-injection of HP DHA demonstrate elevated levels of vitamin C in TRAMP compared to the normal control.

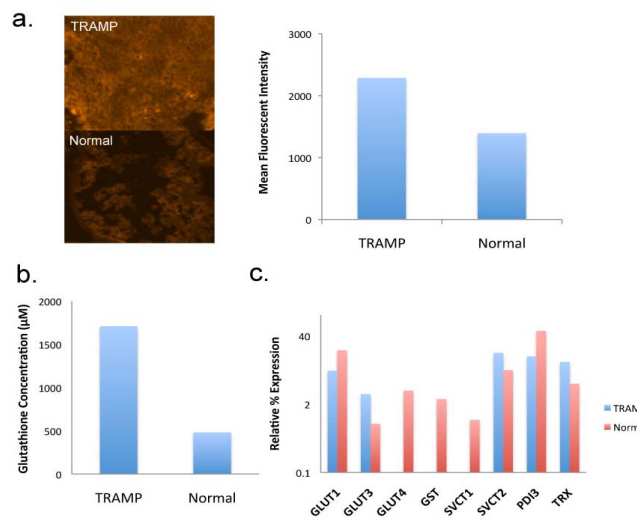
**METHODS: 3T MR Studies:** *In vivo* MRSI studies using HP [1-<sup>13</sup>C] DHA were performed as previously described [1]. **Data Processing and Analysis:** *In vivo* MRSI data was processed using custom software written in IDL 8 (ITT Visual Information Solutions, CO, USA) and Matlab 2009b (MathWorks, MA, USA). DHA and Vitamin C resonance integrals were used to calculate relevant ratios. Average metabolite ratios (VitC/ [VitC + DHA]) were calculated for voxels corresponding to both tumor and surrounding benign tissues in TRAMP mice (n=3). **Mercury Orange Staining and Fluorescent Microscopy:** 8 μm tissue sections from TRAMP mice (n=3) and a normal mouse (n=1) were immersed in 50 μM mercury orange in toluene and rinsed with toluene after 4 minutes then mounted. Slides were viewed by dark-field, phase-contrast transmission fluorescence on a Nikon Eclipse Ti microscope (Nikon, NY, USA) with excitation frequency of 450–490 nm and emission filter barrier frequency of 600 nm. Mean fluorescent intensity was measured in 3 separate fields and averaged using NIS-Elements software (Nikon, NY, USA). **GSH Measurement:** Tissue samples were homogenized in PBS with 0.04% EDTA and assayed with a 5,5'-dithio-bis-2-(nitrobenzoic acid) based absorbance assay (Cayman, MI, USA). **Real-Time PCR:** PCR primers were obtained from Applied Biosciences (Life Technologies, CA, USA), and real-time PCR was performed on total RNA isolated from frozen tissue extracts of TRAMP mice (n=3) and a normal mouse prostate cell line (n=1).

**RESULTS AND DISCUSSION:** TRAMP tumors (n=3) demonstrated elevated ratios of HP vitamin C to DHA (VitC/[VitC + DHA] = 0.23 ± 0.03) compared to surrounding normal tissues and benign prostate (VitC/[VitC + DHA] = 0.06 ± 0.03) (Figure 1). The level of non-protein thiols observed on mercury orange staining was elevated both qualitatively and quantitatively (Figure 2a); mean fluorescent intensity averaged over three ROIs was 2284 ± 265 in TRAMP tumors (n=3) versus 1394 in normal prostate (n=1). Intracellular glutathione levels were significantly elevated in TRAMP prostate (1707 μM ± 74, n=3) compared to normal prostate (481 μM, n=1) (Figure 2b). The relative expression of GLUT1 and GLUT4 was decreased in TRAMP (8.7 and 0 relative % expression, respectively) compared to normal (21.2 and 3.6 relative % expression, respectively), but GLUT3 and thioredoxin reductase expression levels were increased (3.1 and 12.7 relative % expression, respectively, compared to 0.8 and 4.9) (Figure 2c).

**CONCLUSIONS:** The purpose of this study was to further elucidate the mechanism of HP <sup>13</sup>C DHA reduction observed in MRSI studies, with respect to transport, concentration of reducing agent (glutathione), and expression of enzymes that maintain intracellular redox. Surprisingly, analysis of GLUT transporter expression using RT-PCR revealed decreased GLUT1 and GLUT4 mRNA transcripts in TRAMP tumors, although GLUT3 mRNA transcripts were elevated. Future studies will focus on direct interrogation of GLUT transporter proteins (such as by Western Blotting) as mRNA transcripts may not accurately reflect membrane transporter density. Glutathione levels in TRAMP tumors were elevated both by mercury orange staining and by assay of homogenized tissue. This correlation of elevated HP vitamin C to DHA ratios in TRAMP tumors with elevated glutathione suggests that HP DHA MRSI is a noninvasive method to assess levels of GSH. This is significant because elevated GSH levels are correlated with therapeutic resistance in tumors. Finally, analysis of key redox proteins with RT-PCR revealed elevated thioredoxin reductase mRNA, which is significant given findings of elevated thioredoxin reductase in more aggressive lung cancer phenotypes [5].

**REFERENCES:** [1] Keshari *et al.*, PNAS 2011; epub ahead of print. [2] Linster *et al.* 2007, *FEBS J* 274(1):1-22. [3] Blair SL *et al.*, *Cancer Res* 1997;57:152–5. [4] Britten RA *et al.*, *Int J Radiat Oncol Biol Phys* 1992;24: 527–31. [5] Ceccarelli *et al.*, *Int. J. Cancer* 2008;123, 1770–1778.

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**Figure 2.** (a) Mercury orange staining of TRAMP and normal prostate demonstrate elevated levels of non-protein thiols; mean fluorescent intensity averaged over three ROIs was higher in TRAMPs than normal. (b) Intracellular glutathione levels were higher in TRAMP prostate compared to normal prostate. (c) Relative % expression of GLUT 1,3,4 transporters, glutathione-S-transferase, Na<sup>+</sup> channel 1 and 2, protein disulfide isomerase, and thioredoxin reductase in TRAMP tumors versus normal prostate.