**Title:** Hyperpolarized *In vivo* pH Imaging Reveals Grade-Dependent Acidification in Prostate Cancer

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**Abstract Highlights:** We performed hyperpolarized imaging of pyruvate-to-lactate conversion and interstitial pH in a murine prostate cancer model, the TRAMP mouse. Our results demonstrate a significantly lower pH in aggressive versus benign cancer and implicate MCT4-dependent lactate export as an acidification mechanism in this model.

**Introduction:** There is a strong clinical need to noninvasively distinguish benign versus aggressive prostate cancer (PCa). Hyperpolarized (HP) imaging has demonstrated higher lactate conversion from pyruvate and suggested a concomitant decrease in interstitial pH ($pH_e$), although the latter has never been measured. In this study, we investigated whether aggressive and benign PCa in transgenic mice would demonstrate a significant difference in $pH_e$.

**Methods:** Transgenic adenocarcinoma of the mouse prostate (TRAMP) mice were imaged at 14 T using sequences for $^1$H apparent diffusion coefficient (ADC) mapping, $^1$H $T_2$-weighted anatomical imaging, HP [1-$^{13}$C]pyruvate frequency-selective 3D gradient-spin echo imaging, and HP [$^{13}$C]bicarbonate 2D chemical shift imaging. Within 48 hours of imaging, mice were euthanized for tissue collection. Tumor tissue was H&E-stained for histology and used to quantify monocarboxylate transporter 4 (*Mct4*) gene expression via RT-PCR. A trained pathologist classified each lesion as low- or high-grade based upon cell differentiation, glandular pattern, and necrosis. Imaging voxels containing 50% tumor or more were classified as low-grade or high-grade based upon histology. Voxels with a signal-to-noise ratio < 3 or $^1$H ADC value > $3x10^{-3}$ $mm^2/s$ were excluded. Non-parametric Mann-Whitney U-tests and Spearman regression were used.

**Results:** HP imaging was able to resolve metabolic differences between lesions demonstrating low- versus high-grade phenotypes by histology (figure A-D). High-grade lesions (n = 7) showed a higher Lac/Pyr ratio and a lower mean $^1$H ADC and mean/minimum $pH_e$ compared with low-grade lesions (n = 5, figure E-H). Only one low-grade lesion had a $pH_e$ overlapping with the high-grade lesions, demonstrating very good separation based on $pH_e$. Mean lesion $pH_e$ and *Mct4* expression showed a very strong negative correlation (figure I), suggesting that lactate-$H^+$ co-export via MCT4 contributes to greater acidity (figure J).

**Conclusions:** *In vivo* hyperpolarized imaging of pyruvate-to-lactate conversion and $pH_e$ can be performed in a single imaging session. We observed a lower $pH_e$ along with higher lactate in high-grade lesions, suggesting that interstitial acidification may be a biomarker of PCa indolent-to-aggressive transition and is linked with lactate metabolism and export. Future studies will investigate the role of $pH_e$ and lactate in predicting immuno-therapeutic efficacy.