Role of Hypoxia in Aggressive Prostate Cancer – A Hyperpolarized 13C MR Study

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Target audience: Investigators of tumor metabolism, hypoxia, and progression.

Purpose: Like many solid tumors, hypoxia develops in human prostate cancer and is believed to play a key role in promoting tumor progression and poor response to therapy[1]. There is also evidence that the hallmark up-regulation of tumor glycolysis and lactate production is in part a response to the increasingly hypoxic tumor microenvironment with disease progression[2]. Increased tumor lactate production and its excretion leads to a acidic tumor microenvironment that promotes tumor progression through its toxicity to normal cells, degradation of the extracellular matrix by proteinases, increased angiogenesis through the release of VEGF, and inhibition of immune response to tumor antigens[2]. This interplay between cancer metabolism and microenvironment during tumor progression can be uniquely imaged using a multi-probe hyperpolarized (HP) ^{13}C MRI approach[3]. The purpose of this study was to use a combination of 14T ^{1}H multiparametric MRI and multi-probe ^{13}C MR to determine the impact of tumor hypoxia on prostate cancer progression using the TRAMP model of prostate cancer. The TRAMP model was selected since it nicely mimics changes in pathology and metabolism of human disease.

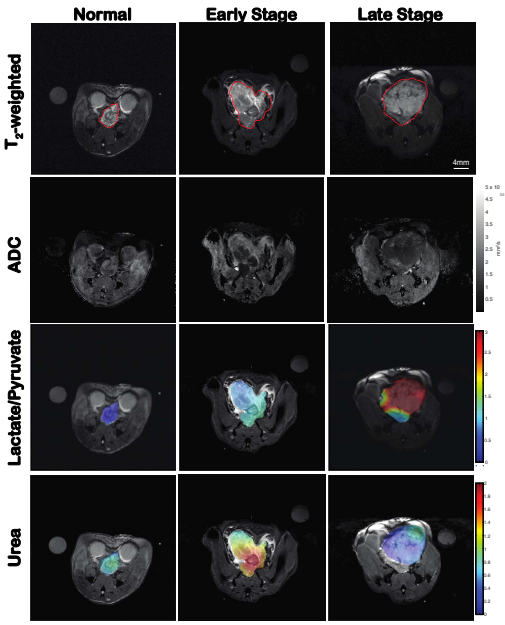

Figure 1. Representative T_2-weighted MR images, ADC maps, ratio maps of [1-^{13}C]lactate to [1-^{13}C]pyruvate and hyperpolarized ^{13}C-Urea distribution in normal prostate, early and late stage TRAMP tumor.

Methods: Based on histopathology, mice were categorized into 3 groups - normal prostate (n=6) early stage tumors (n=6, ≤ 50% poorly differentiated) and late stage tumors (n=9), ≥ 50%) (Fig. 2A). Imaging studies were performed on a wide-bore 14T microimaging system (Varian Instruments) equipped with 100 G/cm gradients and multinuclear capabilities. Multiparametric ^{1}H MR imaging was comprised of T2-weighted anatomic images and diffusion weighted scans to obtain apparent diffusion constant maps (ADC). For the hyperpolarized experiments, [1-^{13}C]pyruvate was co-polarized and injected with ^{13}C Urea[3]. ^{13}C bicarbonate was polarized and injected separately in the same exam. HP ^{13}C MRI was acquired using a frequency specific 3-D GRASE imaging sequence. Normal prostate and TRAMP tumors underwent H&E staining (pathology), KI-67 (proliferation), and pimonidazole (PIM, hypoxia). Additionally, mRNA expression analyses of genes key to the observed metabolic and micro-environmental changes were performed.

Results and Discussion: T_2-weighted images (Fig.1) demonstrate both the contrast and size difference when progressing from the normal prostate (0.04 ± 0.01cc) to early (0.27 ± 0.07cc) and late stage tumors (3.39 ± 0.80cc). Like the human situation, early stage tumors demonstrated both areas of normal glandular structure and regions where the ducts were replaced by cancer cells, whereas, late stage disease exhibited sheets of poorly differentiated cells. These changes in cellularity were reflected in progressive decreases in ADC with disease progression (Figs. 1 & 2B). The magnitude of the ADC changes with cancer grade were very similar to those reported in prostate cancer patients[4]. Metabolically, the lactate to pyruvate (Lac/Pyr) ratio was significantly increased with progression from normal (0.39 ± 0.04) to early (0.83 ± 0.07) and late stage (2.13 ± 0.18) disease (Figs. 1 & 2C). While the normal prostate was non-proliferative and normoxic, there was an increase in both proliferation and hypoxia in early stage disease, and a more dramatic increase in late stage disease (Fig. 2A). Cells in late stage tumors were > 90% proliferative and nearly 20% of the tissue was hypoxic. This relatively large increase in hypoxia in late stage disease resulted in a 2-fold increase in HIF-1α and VEGF and ≈ 4 fold increase in LDHα levels relative to normal and early stage tumor (Figure 2C). MCT4, the transporter primarily responsible for lactate efflux from the cell and extracellular acidification was also increased by 7 fold in late versus early stage cancer, resulting in a acidic interstitial pH measured using hyperpolarized bicarbonate[5] in advanced disease (6.92 ± 0.21) relative to both normal prostate (7.35 ± 0.08) and early stage disease (7.30 ± 0.10). These findings and the strong correlations between increased HIF1α expression and the expression of LDHα (0.97), MCT4 (0.74) and VEGF (0.8) in late stage disease suggest that hypoxia plays a significant role in driving metabolic and micro-environmental changes associated with prostate cancer progression.

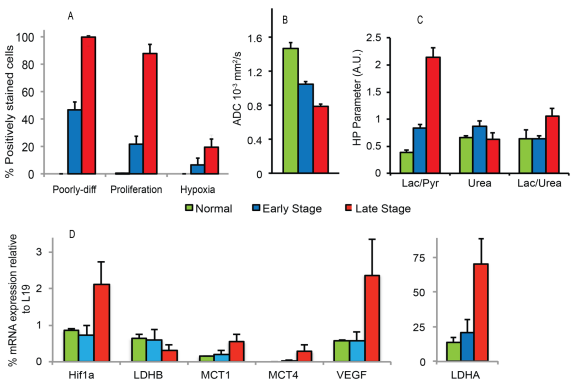

Figure 2. Quantification of A) differentiation based on H&E, proliferation based on Ki67 and hypoxia based on PIM staining, MR parameters B) ADC in the tumor and normal prostate and C) hyperpolarized metabolites (a.u). mRNA expression of relevant genes of hypoxia and metabolism is shown in D.

Conclusions: This study demonstrates that hypoxia is significantly increased in TRAMP tumors, and is responsible for driving metabolic and micro-environmental changes that favor disease progression. Moreover these metabolic and micro-environmental changes can be imaged using a multi-probe hyperpolarized ^{13}C MRI approach. These pre-clinical findings recapitulate the human situation where increasing levels of hypoxia have been measured with increasing clinical stage, and correlated with poor clinical outcomes[6]. A non invasive measurement of hypoxic aggressive disease would also be clinically invaluable as it's a major impediment to radiation therapy, where patients with high grade hypoxic tumors are three times more resistant to radiation therapy[7].

References: (1) Stewart, G. D. et al. *BJU International* **2010**, *105*, 8–13. (2) Gatenby, R. A. and Gillies, R. J. *Nat Rev Cancer* **2004**, *4*, 891–899. (3) Wilson, D. M. et al. *J Magn Reson* **2010**, *205*, 141–147. (4) Nagarajan, R. et al. *Advances in Urology.* **2012**, 2, 1-5. (5) Gallagher, F. A. et al. *NMR Biomed.* **2011**, *24*, 1006–1015. (6) Movsas, B. et al. *Cancer* **2000**, *89*, 2018–2024. (7) Marignol, L. et al. *Cancer Treat Rev* **2008**, *34*, 313–327.

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