Tumor pH and Vascularity in Human Prostate Cancer Models

Ellen Ackerstaff[1], Natalia Kruchevsky[1], Radka Stoyanova[2], Sean D. Carlin[1], Nerissa Viola-Villegas[1], Kuntalkumar Sevak[1], Jason S. Lewis[1], and Jason A. Koutcher[1]
[1]*Memorial Sloan Kettering Cancer Center, New York, NY, United States,* [2]*Miller School of Medicine University of Miami, Miami, FL, United States*

Introduction: Tumor hypoxia and pH have been related to tumor aggressiveness, treatment response, and outcome[1]. Here, we evaluated tumoral pH, metabolism, and vascularity in three preclinical human prostate cancer models of different aggressiveness, using ^{1}H-decoupled ^{31}P MR spectroscopy and dynamic contrast-enhanced MR imaging (DCE-MRI). Tumoral, extracellular pH (pHe) was measured using 3-aminopropylphosphonate (3-APP), while spatial heterogeneity of tumor hypoxia was evaluated from DCE-MRI data using a novel pattern recognition approach.

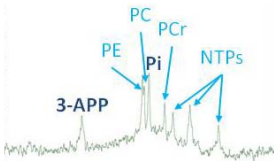

Figure 1: Representative ^{1}H-decoupled ^{31}P MR spectrum of a PC3—CA-IX tumor after 3-APP injection.

Material and Methods: We studied tumors (100 – 300 mm^3) from the three human prostate cancer cell lines, LNCaP, PC-3, and a carbonic anhydrase IX-overexpressing clone of PC-3 (PC-3-CA-IX). Tumors were implanted subcutaneously either on the flank or the shoulder of athymic nude mice. The MR experiments were performed using a custom-built, solenoid ^{31}P MR coil inserted in a ^{1}H MR coil on a horizontal-bore Bruker 7T magnet. A tail vein catheter was inserted, facilitating the administration of 3-APP and Gd-DTPA via a home-built catheter line assembly during the MR experiment. For the duration of the MR experiment, the mice anesthetized via a face mask with < 2% isoflurane in 100% oxygen. The breathing rate, monitored using a pressure sensor, was kept at 50-80 breath/min by adjusting the isoflurane level. The rodent core temperature is maintained at 34-37°C using an MR-compatible, small rodent Heater System. After tuning and matching of the ^{1}H and ^{31}P MR coils, the water line width across the tumor is optimized to ~35-80 Hz full-width-half-maximum using field map-based shimming. Pre-3-APP administration, two consecutive baseline ^{1}H-decoupled ^{31}P MR single pulse spectra averaged over 17 min each are acquired using a 60° excitation pulse, 2 s relaxation delay, 10 kHz spectral width, 2048 points, and 512 averages. Following the baseline spectra, two additional ^{1}H-decoupled ^{31}P MR spectra are acquired with the same parameters, albeit directly before each spectral acquisition, a bolus of 480 mg/kg 3-APP is injected i.v. via the tail vein catheter (**Fig. 1**). For processing, the free induction decays (FIDs) of the two baseline spectra and the two spectra post 3-APP administration respectively are added up, resulting in two 34 min MR spectra (1 pre and 1 post 3-APP administration). An exponential line broadening of 20 Hz is applied to each FID, followed by Fourier transformation of the FID and phase correction of the spectra. The processed MR spectra are exported to Microsoft Excel, the chemical shift of the α-NDP/α-NTP signal calibrated to -10.05 ppm, and an intensity correction applied to the Pi and 3-APP signal[2,3]. As described previously in detail[2], the intracellular and extracellular pH (pHi and pHe) tumoral distributions are calculated from chemical shifts of Pi and 3-APP respectively, using the respective Henderson-Hasselbach equations relating pH and observed chemical shift of Pi or 3-APP (relative to the chemical shift of the α-NDP/α-NTP signal). DCE-MRI data are acquired after the ^{31}P MRS using a FLASH sequence with 15 mm x 15 mm x 4-5 mm field-of-view, 128 x 128 matrix size, 4-5 slices of 1 mm each, as described in detail previously. DCE-MRI data are analyzed using a novel pattern recognition approach, as described by Stoyanova *et al.*[4]. Briefly, principal component analysis (PCA) followed by constrained non-negative matrix factorization (cNMF) allows the estimation of the spatial distributions of tumor perfusion, hypoxia, and necrosis *in vivo*, based on vascularity[4]. For *ex vivo* analysis, representative tumors were excised after the injection of the perfusion marker Hoechst 33342 and the hypoxia, immunohistochemical marker pimonidazole (60 mg/kg). Images of stained sections of the fresh-frozen tumors were captured, depicting perfusion (Hoechst 33342, blue), hypoxia (pimonidazole, green), CA-IX expression (cG250 antibody, red) and necrosis (hematoxylin/eosin).

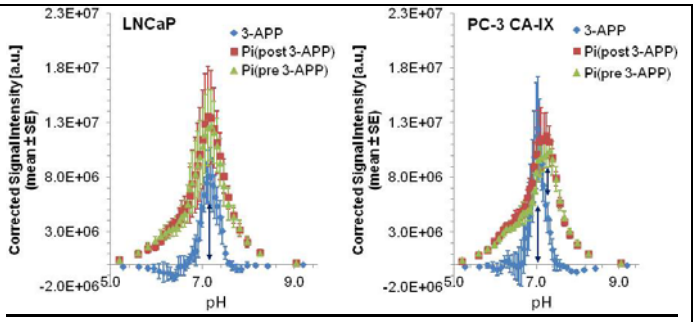

Figure 2: pH distribution of pHe (3-APP) and pHi pre and post 3-APP injection (Pi(pre3-APP) and Pi(post3-APP) respectively) in LNCaP and PC-3-CA-IX tumor xenografts. LNCaP averaged over 4 tumors (pHe, n= 3, one tumor without 3-APP uptake), and PC-3-CA-IX averaged over 5 tumors (pHe, n = 4, one tumor without detectable 3-APP uptake).

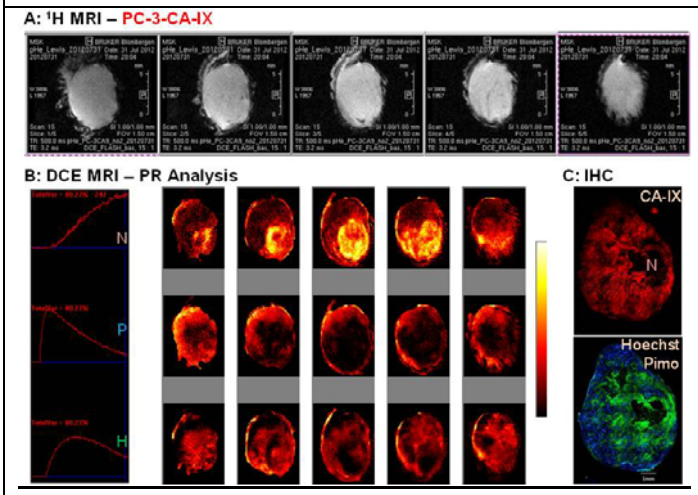

Figure 3: Representative PC-3-CA-IX tumor (168 mm^3 by caliper) (**A**) ^{1}H MR images of the tumor. (**B**) From DCE-MRI data, PR/cNMF identified three contrast agent uptake patterns (N, P, H) and their spatial weighted distributions across the tumor (color scale: white to black, maximum to zero weighting). (**C**) *Ex vivo* analysis of a representative 8 µm section of the tumor depicting the spatial distribution of CA-IX (red), perfusion (blue, Hoechst), and hypoxia (green, pimo).

Results: The resulting pHi and pHe distributions are shown for two different tumor lines in **Fig. 2**. The distribution of pHi appears not to be affected by the dose of 3-APP injected. The overall pHe of PC-3-CA-IX tumors appears to be slightly more acidic than the pHe of LNCaP tumors. Metabolic profiles depicting NTPs and phospholipid metabolites will be presented for the three tumor models. As depicted in **Fig. 3B**, PR/cNMF analysis of the DCE-MRI data found – based on the contrast agent uptake patterns – areas of necrosis (N), high vascular perfusion and permeability (P), and hypoxic areas (H) that qualitatively match the *ex vivo* findings (**Fig. 3C**).

Conclusion: Our preliminary results indicate that in tumors without extensive necrosis, pHe appears to be only slightly acidic and tied to the extent of tumoral hypoxia as determined *in vivo* from DCE-MRI data.

References: 1. Vaupel, P., Semin Radiat Oncol, 2004. 14(3):198-206. 2. Raghunand, N., Methods Mol Med, 2006. 124:347-64. 3. Graham, R.A., *et al.*, Am J Physiol, 1994. 266(2 Pt 2):R638-45. 4. Stoyanova R., *et al.* Transl Oncol 2012, *in press*.

Acknowledgments: Supported by NIH grants PO1 CA115675, R01 CA138468, R24 CA83084, NCI P30 CA0874 (Cancer Center Support Grant), and in part by GEMS. We like to thank Dr. D.C. Shungu and X. Mao for the software XsOsNMR.