Assessment of the Tumor Type-Specific Microenvironment – Lactate, Vascularity, Hypoxia, Extracellular pH
Ellen Ackerstaff1, Natalia Kruchevsky1, Ekaterina Moroz1, Niralanto Ramamonjisoa1, Rui V. Simoes1, H. Carl LeKaye1, Kristen L. Zakian1, Hansol Lee2, HyungJoon Cho3, Radka Stoyanova1, Inna Senganova1, Ronald G. Blasberg1, and Jason A. Koutcher1
1Memorial Sloan-Kettering Cancer Center, New York, NY, United States, 2Ulsan National Institute of Science and Technology, Ulsan, Korea, 3Miller School of Medicine, University of Miami, Miami, FL, United States.

Target Audience: Our in vivo study is of great interest to cancer researchers who investigate the impact of the abnormal tumor microenvironment on tumor growth, progression, metastasis, and treatment resistance. More aggressive tumors have been associated with increased lactate production and acidity, contributing to a suppressed T-cell immune response. Here, we characterize noninvasively in vivo the tumor microenvironment in 5 tumor models of different origin and aggressivity and investigate the relationship of lactate metabolism, vascularity, hypoxia, and extracellular pH (pHe) to tumor type / aggressivity.

Methods: Tumor Models: We studied 4 prostate cancer (CaP) cell lines – LAPC-4 (human advanced prostate adenocarcinoma, kindly provided by Dr. Sawyer1), MycCaP (spontaneously immortalized cells from C-Myc transgenic mouse with CaP, androgen naïve), PC-3 (bone metastasis of human grade IV prostate adenocarcinoma), RM-1 (CaP of Ras+Myx-transformed C57BL/6 mouse) – and a tumorogenic, human embryonic kidney cell line (HEK). All cell lines were grown in Dulbecco’s Modified Essential Medium (MEM), supplemented with 10% fetal bovine serum, 100 U/ml Penicillin and 100 μg/ml Streptomycin at 37 °C in 5% CO2. Cancer cells were injected subcutaneously in the right flank of Nod/SCID mice (Jackson Laboratory). In Vivo MR: The MR experiments were performed using a custom-built, solenoid 1H MR coil on a horizontal-bore Bruker 7T magnet. A tail vein catheter was inserted, facilitating the administration of Gd-DTPA and pH marker ISUCA (Soirem Research Ltd.) via a home-built catheter line assembly. During the MR experiment, mice were anesthetized with < 2% isoflurane in oxygen. The breathing rate was kept at 50-90 breath/min by adjusting the isoflurane level. The rodent core temperature was maintained at 34-37°C. After tumor positioning, the 1H MR coil was tuned and matched; the line width of tumoral water was centered by 60-70 Hz full-width-half-maximum (FWHM)-based shimming. For each tumor, we evaluated the lactate distribution, tumor vascularity, and, where applicable, pHe (Fig. 1). Tumoral lactate levels were assessed using Sel-MQC. Single-slice MRSI data were acquired with 16 mm x 16 mm field-of-view (FOV), 8x8 matrix, 3 s TR, 120 ms TE, with slice thickness varied to cover the entire tumor. Data were processed and lactate signals fitted using XsOsNMR. Absolute quantification of lactate levels, as described previously, is in process. Following lactate MRSI, tumor vascularity was assessed by dynamic contrast enhanced (DCE)-MRI as described previously. Briefly, DCE-MRI data were acquired using FLASH with 15 mm x 15 mm FOV, 128×128 matrix, 5 slices of each mm 1 each. DCE-MRI data are being analyzed using principal component analysis (PCA) followed by constrained non-negative matrix factorization (cNMF), thus, estimating the spatial distributions of tumor perfusion, hypoxia, and necrosis in vivo based on vascularity. Extracellular pH (pHe) was assessed using ISUCA as described previously with modification to adapt for tumor site. Briefly, after a baseline single-voxel 1H MR PRESS spectrum of a non-neurotic tumor region, ISUCA was infused at 0.6 mmol/kg for 20 min, followed by 0.4 mmol/kg for 90 min. For tumors with significant, detectable ISUCA, 1H MRSI was acquired. The data were processed and the chemical shift of the H2 ISUCA resonance in reference to total choline at 3.2 ppm obtained, using XsOsNMR or MestReNova. The pHe was calculated using the ISUCA-specific Henderson Hasselbalch equation: pHe = 7.07 + log[(8.74595)/(5.76779)]. In poorly perfused tumors, ISUCA was not detectable and pHe could not be obtained.

Results & Discussion: HEK tumors were characterized by high levels of lactate across the tumor (Fig. 1A, Fig. 2), and moderate acidic pHe (Fig. 1D, Fig. 2). As in HEK tumors (Fig. 1B), LAPC-4 and MycCaP tumors were heterogeneously vascularized, with an interplay of areas depicting Gd-DTPA uptake representative of well-vascularized, hypoxic, and necrotic areas. For those tumors, well-enough vascularized to assess pHe, only trends and no notable differences between tumor types were seen (Fig. 2) – probably due to too small sample size (n = 2 to 3). Of the five tumor models studied, PC-3 tumors had often already at small tumor sizes (<200 mm3) significant central necrosis with a perfused tumor rim, resulting in lower or undetectable tumor lactate (Fig. 2). RM-1 tumors had variable lactate content and too little vascularity to obtain their pHe (data not shown). Our data suggest that pHe does not relate directly to tumor lactate levels (Fig. 1A, D). Our data suggest also that interplay of cancer cell metabolic activity and vascularity, as well as the interplay of the tumor microenvironment and phenotype to adaptive T-cell therapy. Our long-term goal is to assess metabolic activity, vascularity, and pHe by independent measurements.

Conclusion/Outlook: Our in vivo study is of great interest to cancer researchers who investigate the impact of the abnormal tumor microenvironment on tumor growth, progression, metastasis, and treatment resistance. More aggressive tumors have been associated with increased lactate production and acidity, contributing to a suppressed T-cell immune response. Here, we characterize noninvasively in vivo the tumor microenvironment in 5 tumor models of different origin and aggressivity and investigate the relationship of lactate metabolism, vascularity, hypoxia, and extracellular pH (pHe) to tumor type / aggressivity.


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Figure 1: Representative data from a HEK flank tumor (276 mm3) by 1H MRSI. (A) Lactate MRSI; (B) well-vascularized (P), hypoxic (H), and necrotic (N) tumor areas by DCE-MRI; (C) Single-Voxel 1H PRESS MRS (66.5 mm3, from bottom to top: before, 16, 32, and 48 min after start of ISUCA infusion); (D) ISUCA MRSI: Left: Tumoral ISUCA distribution; Right: pHe map.

Figure 2: Scatter plots and corresponding means ±SD of tumor type-specific lactate levels and pHe. Red data point depicts tumor shown in Fig. 1.