Assessment of the Tumor Type-Specific Microenvironment – Lactate, Vascularity, Hypoxia, Extracellular pH

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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 response.

Purpose: A hostile tumor microenvironment, characterized by vascular abnormalities, hypoxia, and low pH, impacts tumor growth, progression, metastases, and treatment resistance[1]. More aggressive tumors have been associated with increased lactate production and acidity[1], contributing to a suppressed T-cell immune response[2]. 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. Sawyer[3]), MycCaP (spontaneously immortalized cells from C-Myc transgenic mouse with CaP, androgen naïve[4]), PC-3 (bone metastasis of human grade IV prostate adenocarcinoma[5]), RM-1 (CaP of Ras+Myc-transformed C57BL/6 mouse[6]) – and a tumorigenic, 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% CO_2. 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 ^{1}H MR coil on a horizontal-bore Bruker 7T magnet. A tail vein catheter was inserted, facilitating the administration of Gd-DTPA and pHe 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 ^{1}H MR coil was tuned and matched; the line width of tumoral water was optimized to ~30-70 Hz full-width-half-maximum by field map-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[7]. 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[8], is in process. Following lactate MRSI, **tumor vascularity** was assessed by dynamic contrast enhanced (DCE)-MRI, as described previously[9]. Briefly, DCE-MRI data were acquired using FLASH with 15 mm x 15 mm FOV, 128x128 matrix, 5 slices of 1 mm each. DCE-MRI data are 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[10]. **Extracellular pH (pHe)** was assessed using ISUCA as described previously[11] with modification to adapt for tumor site. Briefly, after a baseline single-voxel ^{1}H MR PRESS spectrum of a non-necrotic 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, ^{1}H MRSI was acquired[11]. The data were processed and the chemical shift δ of the H2 ISUCA resonance in reference to total choline at 3.2 ppm obtained, using XsOsNMR or MestRecNova. The pHe was calculated using the ISUCA-specific Henderson Hasselbalch equation[11]: pHe = 7.07 + log[(8.7459-δ)/(δ-7.679)]. In poorly perfused tumors, ISUCA was not detectable and pHe could not be obtained.

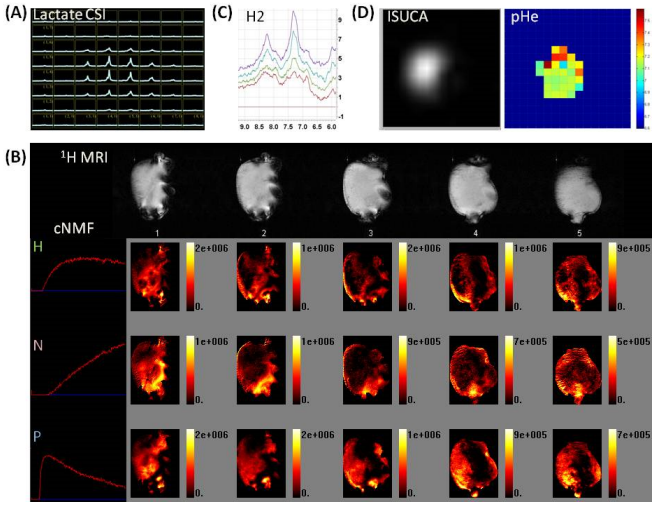

Figure 1: Representative data from a HEK flank tumor (276 mm^3 by ^{1}H MRI). **(A)** Lactate MRSI; **(B)** well-vascularized (P), hypoxic (H), and necrotic (N) tumor areas by DCE-MRI; **(C)** Single-Voxel ^{1}H PRESS MRS (66.5 mm^3, from bottom to top: before, 16, 32, and 48 min after start of ISUCA infusion); **(D)** ISUCA MRSI: Left: Tumoral ISUCA distribution; Right: pHe map.

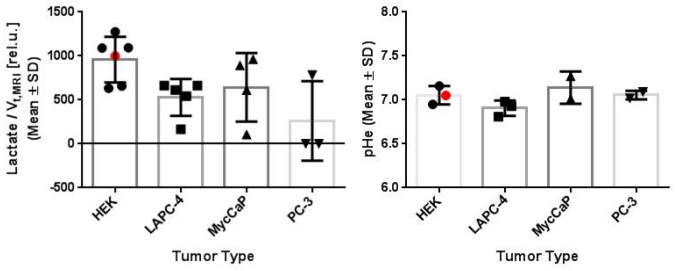

Figure 2: Scatter plots and corresponding means (±SD) of tumor type-specific lactate levels and pHe. Red data point depicts tumor shown in **Fig. 1.**

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 mm^3) 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 vascularization 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 vascularization regulates tumoral pHe, showing the importance to assess metabolic activity, vascularization, and pHe by independent measurements.

Conclusion/Outlook: Our long-term goal is to assess in CaP the contribution of the tumor microenvironment and phenotype to adaptive T-cell therapy.

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