Tumor pH and Vascularity in Human Prostate Cancer Models
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Introduction: Tumor hypoxia and pH have been related to tumor aggressiveness, treatment response, and outcome1. Here, we evaluated tumor pH, metabolism, and vascularity in three preclinical human prostate cancer models of different aggressiveness, using 1H-decoupled 31P 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.

Material and Methods: We studied tumors (100 – 300 mm3) from 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 31P MR coil inserted in a 1H 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 1H and 31P 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 1H-decoupled 31P MR single pulse spectra acquired over 17 min each are acquired using a 60° excitation pulse, 2 s relaxation delay, 10 kHz spectral width, 2048 points, and 512 averages. Two additional 1H-decoupled 31P 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 acquired 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 signal2,3. As described previously in detail2, 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 31P 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 necrosis, hypoxia, and necrosis in vivo, based on vascularity5. 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).

Results: The resulting pH and pHe distributions are shown for two different tumor lines in Fig. 2. The distribution of pH does not appear 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 NTDP 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 that 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.


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