Characterization of the Tumor Microenvironment using combined MRI, MRSI and Optical Imaging

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Introduction: The hypoxia inducible factor (HIF-1) mediates the adaptive response of cells to hypoxia. Under normoxic conditions, HIF-1α is subject to ubiquitination and proteasomal degradation. Under hypoxic conditions, ubiquitination of HIF-1α is dramatically reduced and the activity of its transcriptional activation domain increased [1]. HIF-1 can act as a transcriptional activator for several key genes such as VEGF and several glycolytic enzymes. Each of these target genes contains hypoxia response elements (HRE), which include one or more HIF-1 binding sites. By placing GFP expression under the control of an HRE, it is possible to indirectly detect HIF-1 activity and hypoxia in tissues. In these studies, we have derived tumors from a stably transfected human prostate cancer cell line, PC-3, with HRE ligated to GFP, and performed combined MRI, MRSI and optical imaging to obtain co-localized maps of vascular volume, permeability, total choline, lactate/lipid and hypoxia.

Methods: We generated PC-3 tumors derived from cells stably transfected with the hypoxia response element (HRE) of human VEGF-A ligated to the enhanced green fluorescence protein (EGFP) gene. Studies were performed on seven tumors. Vascular and metabolic maps of HRE-GFP PC-3 tumors were obtained on a GE 4.7T and more recently a Bruker Biospec Avance 4.7T spectrometer using MRI for vascular imaging and MRSI for metabolic imaging. Maps of vascular volume and permeability were obtained as previously described [2] using the intravascular contrast agent albumin-GdDTPA, from three or four 1 mm thick slices through the tumor. A small glass capillary containing water doped with GdDTPA was used as a spatial reference, together with the orientation of the head and tail of the animal. Metabolic maps of total choline and lactate/lipid were obtained from a co-localized 3 or 4mm thick slice, with an in-plane resolution of 1mm×1mm using either the BASSALE sequence or a 2D CSI sequence with VAPOR water suppression [3]. After imaging, each tumor was marked for spatial referencing, and sectioned to obtain 1mm thick slices coarsely matching the MR imaged slices. Optical imaging of GFP expression in freshly cut tumor sections was used to visualize hypoxia in relation to the noninvasively obtained MR vascular and metabolic maps.

Results: Examples of co-localized total choline, GFP distribution, combined vascular volume/permeability/total choline, and combined vascular volume/permeability/lactate-lipid are shown in Figure 1-3. Five of the seven tumors examined showed co-localization of GFP with high total choline. High permeability was detected either in or around the GFP and high total choline regions (regions of cyan in Figure 1c). These tumors were relatively small with a mean tumor volume of 178±47 mm^3 and did not exhibit large foci of necrosis. The lactate/lipid signal was usually co-incident with some, but not all, regions of high vascular volume as evident from the areas of magenta in Figure 1d.

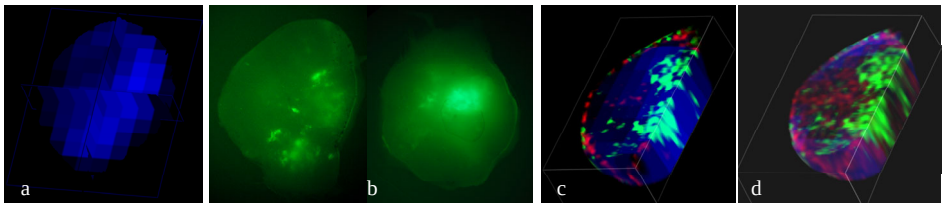

Figure 1: Triplanar or 3D views of 3D reconstructed maps from an HRE-PC-3 tumor of (a) total choline (b) HRE-GFP hypoxia distribution (c) combined vascular volume (red), permeability (green), total choline (blue), and (d) combined vascular volume (red), permeability (green), lactate/lipid (blue). Regions of high metabolite levels and high permeability are apparent as cyan, and regions of high metabolite level and high vascular volume are apparent as magenta. Tumor volume 270mm^3.

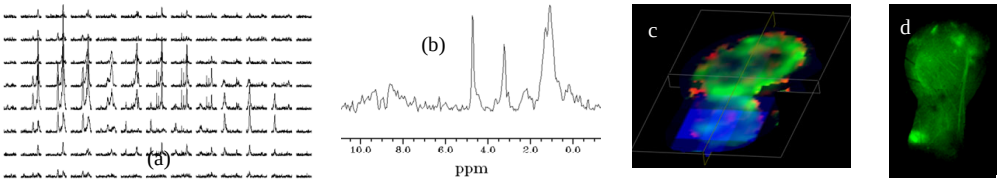

Figure 2: (a) Example of a CSI data set obtained on a Bruker 4.7T system from a 6x3x3 mm^3 tumor. (b) Spectrum from a single 1×1×2mm^3 voxel showing total choline at 3.2ppm and lactate/lipid at 1.3ppm (TE= 272 ms). (c) Triplanar view of vascular volume (red), permeability (green) and total choline (blue) and (d) GFP distribution in the slice. An intense GFP region is apparent in (d) which co-localizes with high total choline in (c).

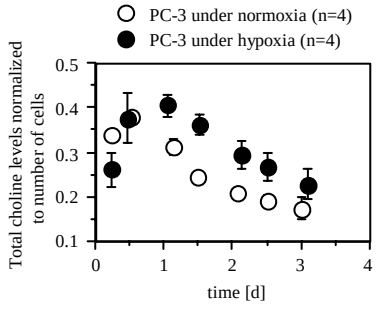

Figure 4. Changes in total choline over time in PC-3 cells maintained under normoxia or hypoxia. The total choline was normalized to the cell number using the signal from intracellular water.

Discussion: These *in vivo* data are consistent with our cell perfusion studies with the PC-3 cell line under normoxic and hypoxic conditions, where hypoxia increased the total choline signal of intact cells (Figure 4). These data suggest that hypoxia may drive, in part, the high total choline observed in tumors.

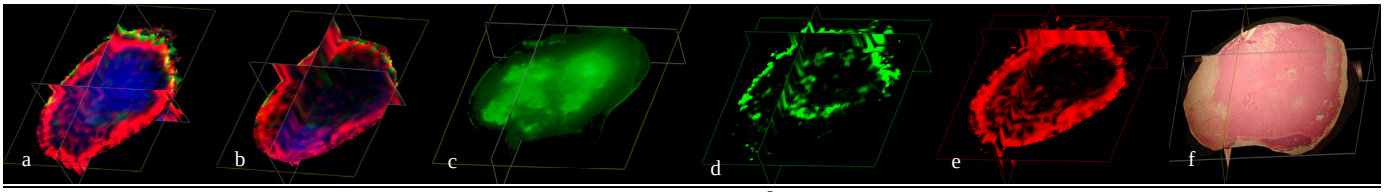

Figure 3: Triplanar views of 3D reconstructed maps from an HRE-PC-3 tumor (172mm^3) of (a) combined vascular volume (red), permeability (green), total choline (blue), (b) combined vascular volume (red), permeability (green), lactate/lipid (blue), (c) HRE-GFP hypoxia distribution, (d) permeability map in green, (e) vascular volume map in red, (f) H&E stained sections. In this tumor, high permeability was detected surrounding the region of high GFP expression, whereas high total choline was detected within this region. Vascular volume was very low in the high GFP expressing region.

References: 1. Semenza GL (2000). *Crit Rev Biochem Mol Biol*; 2.Bhujwalla ZM et al (2001). *Neoplasia;* 3.Shungu DC, Glickson, J.D. (1994). *MRM*.

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