The Assessment of Tumor Angiogenesis and Hypoxia Using Multi-parametric and Multi-modal Imaging

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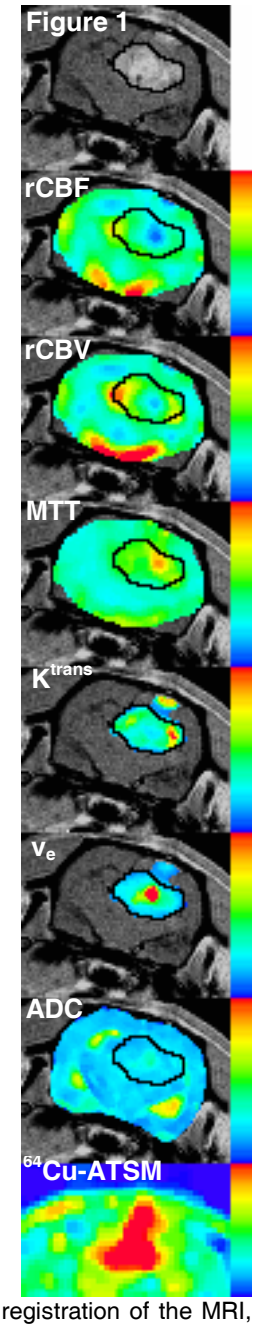
Introduction: Tumor hypoxia can occur when increases in cellular proliferation and metabolic activity are inadequately responded to by the tumor angiogenic response. Despite a substantial increase in the vascular density, the delivery of oxygen to tumor cells can be reduced or even eliminated by longer diffusion distances, structural abnormalities of the developing vessels and inconsistent blood flow patterns. The purpose of this study is to develop a multi-parametric MRI imaging protocol that can non-invasively assess these physiological characteristics and spatially correlate them with ^{64}Cu-ATSM PET images of tumor hypoxia.

Methods: Diffusion Weighted (DWI) MRI, Dynamic Susceptibility Contrast (DSC) MRI and Dynamic Contrast Enhanced (DCE) MRI studies have been performed on 2 C6 glioma inoculated Wistar rats using a 7.0T Varian scanner. Anesthesia was induced using 1.1% isoflurane and core temperature was maintained at 37.5 ± 0.5°C. A common image acquisition scheme was utilized and consisted of a 35 x 35 mm FOV, a 1 mm ST, a 0.5 mm slice gap and 5 slices (center slice was used for the DSC-MRI scan). Diffusion weighted images were collected using 4 b-values: 0, 100, 400 and 800. Maps of ADC, a marker of cellular density, were computed from these images. Maps of relative cerebral blood flow (rCBF), relative cerebral blood volume (rCBV) and mean transit time (MTT) were created using a first pass DSC-MRI study. For this study a GE pulse sequence was used to collect 120 images at a rate of one image per second. At 1 minute into the scan a 8 mg/kg bolus of the intravascular contrast agent, Molday-ION (Biophysics Assay Laboratory, Worcester, MA), was injected through a jugular vein. After the CR-BOLD study a variable flip angle spoiled GE approach was employed to produce pre-contrast (Gd-DTPA) T_1-maps. For the DCE-MRI study 45 serial images were collected at a rate of 51 seconds per image following the injection of Gd-DTPA via a second jugular vein. The reference region model was applied to the DCE-MRI data to produce maps of the volume transfer constant, K^{trans}, and the volume fraction of the extravascular, extracellular space (EES), v_e (2). To register the MRI images to microCT and microPET data, whole brain images were collected using a 3D acquisition. Following the MRI scan, 350-500 μCi of the hypoxia radiotracer, ^{64}Cu-ATSM, was injected into each animal and scanned twenty-four hours later using a Concorde microPET system (2). Finally, for registration purposes anatomical images were acquired using an Imtek microCT scanner. The tumor volume was determined using the region of contrast enhancement on the DCE-MRI images. The hypoxic tumor fraction was determined as the percentage of the tumor showing increased uptake of ^{64}Cu-ATSM.



Results: Figure 1 shows examples of the multi-parametric maps created using this protocol for one slice through the center of the tumor. The top image is a DCE-MRI image after contrast injection and was used to outline (in black) the primary enhancing region of the tumor. While all of the MRI images are registered to each other, the PET image on the bottom was taken from a slice centered through the tumor region showing an increased uptake of ^{64}Cu-ATSM. For the two tumors studied thus far, the hypoxic tumor fractions were 55% (shown in Fig. 1) and 88%. As seen in the DSC-MRI images (rCBF, rCBV, MTT) most of the enhancing tumor region had increased vascularity. The tumor rCBF and rCBV values were, on average, about 1.5 and 2 times greater than that found in contralateral brain tissue. In the tumor containing an 88% hypoxic fraction these values were even higher (2 and 2.5, respectively). However, the MTT values throughout both tumors were much higher than those found in normal brain tissue. The K^{trans}, v_e and ADC maps indicate a core region of necrotic tissue (low K^{trans}, high v_e, high ADC) but in the PET image no such region was found. Since only viable tumor cells can take up the ^{64}Cu-ATSM this demonstrates the sensitivity of this PET method to a small fraction of viable cells in this region. In both tumors, regions of increased K^{trans} and rCBF did not always overlap indicating that K^{trans} is likely reporting on regions of increased permeability in this tumor model. As previously reported the ADC in the tumor periphery was similar to that of normal brain tissue and elevated in the tumor core.

Discussion: This preliminary study and analysis demonstrates the potential of this multi-modal and multi-parametric approach to more thoroughly interrogate the tumor microenvironment. At this late time point in the tumor's growth the MTT was the only parameter that grossly correlated with regions of tumor hypoxia. Normal tissue nMTT could be considered the "ideal" temporal delivery of blood to provide uniform tissue oxygenation. We therefore hypothesize that the higher MTT values observed in these tumors may be indicative of an increased residence time of erythrocytes in the vasculature. This could occur when the resistance through the vascular network has substantially increased as commonly occurs in tumors. In these cases the hemoglobin can be fully deoxygenated before leaving the capillary network. Thus, an increase in the MTT could correspond to a decrease in the tissue perfusion efficiency, and despite an increase in vascular density would likely result in tissue hypoxia. As reported by others, a single time point ADC measurement, like those shown here, does not clearly reveal areas of increased cellular density (i.e. local regions with lower than normal brain tissue ADC values). It is likely, however, that this parameter will prove more useful for measuring changes in cell density over time (e.g. functional diffusion maps) (2). Ongoing studies include increasing the number of animals, 3D registration of the MRI, PET and CT images, voxel-wise statistical comparison of the parameters and a longitudinal study tracking the spatial development of hypoxia, angiogenesis, cellularity, and metabolism (using ^{18}FDG-PET) followed up with histological validation.

References: 1. Yankeelov TE, Mag Res Imag 2005; 23: 519-529. 2. Moffat BA, Proc Natl Acad Sc. 2005; 12;102(15):5524-9.