Water diffusion is disrupted in low collagen containing hypoxic regions of breast cancer xenograft

Samata Kakkad[1,2], Jiangyang Zhang[3], Alireza Akhbardeh[1], Meiyappan Solaiyappan[1], Venu Raman[1], Dieter Leibfritz[2], Kristine Glunde[1], and Zaver Bhujwalla[1]
[1]JHU ICMIC Program, Division of Cancer Imaging Research, The Johns Hopkins University SOM, Baltimore, MD, United States, [2]Department of Chemistry and Biology, University of Bremen, Bremen, Germany, [3]Russell H. Morgan Department of Radiology and Radiological Science, The Johns Hopkins University SOM, Baltimore, MD, United States

Introduction: Tumors display chaotic vasculature that leads to hypoxia. Hypoxia is associated with an aggressive phenotype and increased resistance to radiation and chemotherapy. Hypoxic tumor regions also contain very few collagen 1 fibers [1], which is a major component of the tumor extracellular matrix (ECM) and plays an important role in molecular movement through the ECM. Here we examined the effects of hypoxia on water diffusion in a breast cancer xenograft model engineered to fluoresce under hypoxia. We observed, for the first time, that in hypoxic regions water diffusion and diffusion anisotropy was lower than in normoxic regions that may be explained by the few collagen fibers in hypoxic regions.

Methods: Female severe combined immunodeficient mice were inoculated in the mammary fat pad with $2x10^6$ MDA-MB-231 cells stably expressing red florescence protein (RFP) under the control of a hypoxia response element (HRE). Once tumor volumes were approximately 300-400 mm^3 the tumor was excised and fixed for 20 h in 4% paraformaldehyde, and washed with phosphate buffered saline for 72 h. The sample was spatially marked and placed in a 10- mm NMR tube immersed in Fomblin perfluoro polyether solution (Solvay Solexis). An 11.7 Tesla spectrometer (Bruker Biospin) was used to acquire high-resolution T2- and T2*-weighted MRI. Diffusion tensor imaging (DTI) of this sample was performed in three dimensions (3D) with two non-diffusion weighted images and eight diffusion-weighted images (b=1500 s/mm^2) (resolution 60 x 60 x 60 μm^3). The apparent diffusion coefficient (ADC) and fractional anisotropy (FA) maps were calculated. Following DTI, the tumor was sliced at 1 mm thick sections with an adjustable tissue slicer (Braintree Scientific, Inc, Braintree, MA) for optical imaging. A Nikon inverted microscope (Nikon Ltd., Melville, NY), was used to acquire bright field and fluorescence field optical images to detect the RFP hypoxic regions using a 1x lens to cover the entire tumor section. All analysis was done in MATLAB (Mathworks Inc.). Multimodality registration was performed by feature extraction to co-register the optical images to the DTI images. Statistical dice similarity indices were calculated as the registration error. Hypoxic regions were identified from the optical fluorescence field images and the ADC and FA values were calculated in the corresponding hypoxic and normoxic regions.

Results and Discussion: Heterogeneous ADC and FA value distributions were identified in the DTI data acquired from the tumors (Figures 1a and b). The red fluorescent hypoxic regions were identified from the optical images (Figure 1d). Reregistration strength was confirmed by dice similarity index = 0.88 (Figure 1e), which is a good overlap for multimodality images. The ADC map overlaid the optical image demonstrates reduced ADC in hypoxic regions (Figure 1f). Overall, hypoxic regions had lower water diffusion (ADC) (Figure 1g) and diffusion anisotropy (FA) values (Figure 1h) than normoxic regions. We have consistently observed that Col1 fibers are significantly decreased in hypoxic regions [1], which could explain the lower ADC and FA values observed in hypoxic regions. In separate studies we have also observed high ADC in high collagen fiber containing regions in human breast cancer tissue. The low ADC and FA observed in low collagen containing hypoxic regions indicates a functional role of these fibers in molecular transport. Decreased diffusion of molecules due to few collagen fibers in hypoxic regions may also contribute to poor drug delivery and tumor recurrence in hypoxic regions. Increased cellularity is thought to be one reason why lower ADC and FA values are observed in tumors [2] [3]. An even lower ADC within this backdrop may be due to hypoxic tumor regions.

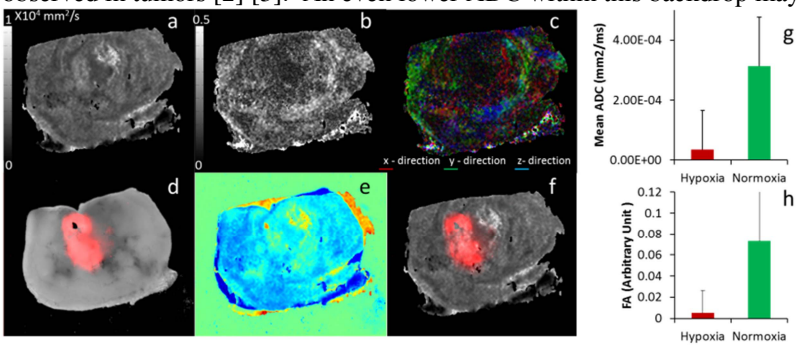

Figure 1: (a) Apparent diffusion co-efficient (ADC) map; (b) fractional anisotropy (FA) map; (c) DTI directions color-map; (d) corresponding registered optical bright field image overlaid with fluorescence field image; (e) ADC map overlaid with registered optical bright field section showing good co-registration; (f) registered hypoxic region overlaid the ADC map; (g) ADC values in hypoxic and normoxic regions showing lower ADC with hypoxia; (h) FA values in hypoxic and normoxic regions showing relatively lower fractional anisotropy with hypoxia.

References: [1] Kakkad et al., Neoplasia, 2010; [2] Riham et al., Radiology, 2010; [3] Partridge et al., J. Magn. Reson. Imaging.

Acknowledgement: This work was supported by NIH P50 CA103175 and P30 CA006973.