In vivo and ex vivo diffusion tensor imaging parameters follow Collagen 1 fiber distribution in breast cancer xenograft model

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Introduction: Collagen 1 (Col1) fibers are a major structural component of the extracellular matrix (ECM) of tumors [1]. Malignant breast cancers are characterized by significantly higher Col1 fiber density and an altered Col1 architecture [1]. Col1 fibers play an important role in molecular transport and cancer cell dissemination. Second harmonic generation (SHG) microscopy is used to detect Col1 fibers but apart from endoscopic applications, optical imaging cannot be easily used as a noninvasive imaging modality due to its limitations of depth penetration. Diffusion tensor imaging (DTI) parameters such as the apparent diffusion coefficient (ADC) and fractional anisotropy (FA), have been used to visualize fiber tracts and quantitatively measure white matter integrity [2]. Analogous to white matter tracts in the brain, Col1 fibers in breast cancer may mediate the extent of water diffusion, and the degree of anisotropy. We have previously observed that hypoxic regions, detected by red fluorescence in an MDA-MB-231 human breast cancer xenograft model engineered to express red fluorescence protein under hypoxia, typically contain fewer Col1 fibers [3, 4]. We therefore characterized diffusion in hypoxic and normoxic regions of these tumors *in vivo* and *ex vivo* to further validate the relationship between Col1 fibers and diffusion, and determine if DTI parameters provided noninvasive indices of Col1 fiber distribution in tumors. We confirmed that hypoxic regions contained significantly fewer Col1 fibers [3], and were characterized by a lower ADC and FA compared to normoxic tumor regions. Diffusion patterns observed *in vivo* were spatially similar to those observed *ex vivo*, suggesting that noninvasive DTI can be used to evaluate Col1 fiber density and potentially hypoxia in tumors.

Methods: $2x10^6$ MDA-MB-231 cells stably expressing red florescence protein under the control of a hypoxia response element [4] were inoculated in the mammary fat pad of female severe combined immunodeficient mice. Once the tumor volumes were approximately 300-400 mm^3, the mice were anesthetized to acquire *in vivo* DTI using a horizontal 11.7T Bruker system with a 10 mm diameter solenoid coil. DTI was acquired with 5 non-diffusion weighted and 30 diffusion directions, (b-value ~1500 s/mm^2, resolution = 105 x 105 μm^2 x 20 z-slices). Following *in vivo* DTI, the tumor was excised and fixed in 4% paraformaldehyde. A vertical 11.7 Tesla spectrometer was used to acquire *ex vivo* three dimensional DTI with two non-diffusion weighted images and eight diffusion-weighted images (b=1500 s/mm^2, resolution 60 x 60 x 60 μm^3). Following *ex* vivo DTI, the tumor was sectioned at 1 mm slice thickness to detect hypoxic regions by fluorescence microscopy using a 1× objective attached to a Nikon microscope. Following the optical image acquisition, sections were paraffin-embedded and sectioned at 5 µm thickness for immunohistochemistry and stained with hematoxylin and eosin (H&E). These H&E sections were used to acquire tiled scan SHG microscopy images to detect the Col1 fiber distributions in 3D (incidence = 860 nm, emission = 410-470 nm) using a 25× lens on an Olympus FV1000 multiphoton microscope. ADC and FA maps were calculated for both *in vivo* and *ex vivo* DTI. Multimodality co-registration was performed using affine transformation to first co-register the *ex vivo* DTI data to *in vivo* DTI data, and then the optical images to the diffusion images. H&E sections were co-registered to corresponding DTI images to detect necrotic regions. Pearson's correlation coefficient was calculated for *in vivo* and *ex vivo* ADC and FA maps. Additional, ADC, FA and Col1 fibers were quantified in normoxic, hypoxic and necrotic regions.

Results and Discussion: We found that high Col1 fiber density correlated with increased apparent diffusion coefficient (ADC) and fractional anisotropy (FA) (**Figures 1 & 2**). Consistent with our previous observations, hypoxic regions contained significantly fewer Col1 fibers [3], and exhibited a lower ADC and FA compared to normoxic tumor regions. In necrotic regions, significantly higher ADC values were observed with low FA values (**Figure 2**). Diffusion patterns observed *in vivo* were spatially similar to those observed *ex vivo*, suggesting that noninvasive DTI can be used to evaluate Col1 fiber density in tumors. The combination of the ADC and FA patterns could be used to predict the tumor microenvironment conditions such as normoxic, hypoxic or necrosis (**Figure 3**). The results obtained here support further investigation of using ADC and FA to noninvasively image Col1 fiber density as well as hypoxia for patient management. Additionally, these data extend our earlier studies, which demonstrated that Col1 fibers facilitate macromolecular transport [5]. The results obtained here suggest that low molecular weight agent transport through the ECM is also mediated by Col1 fibers, with implications for the delivery and transport of low molecular weight chemotherapy agents through the ECM. These results indicate that diffusion MRI may be used to assess Col1 fiber density in breast lesions, which is useful because high Col1 fiber density is a hallmark of malignant breast cancers and can facilitate metastatic dissemination from the primary lesion along Col1 fibers [2]

References: [1] Provenzano P *et al*, BMC Med 2008; [2] Mori S *et al*, Neuron, 2006; [3] Kakkad SM *et al*, Neoplasia, 2010; [4] Krishnamachary B *et al*, PLoS One 2013. [5] Kakkad SM *et al*, PLoS One 2013. **Acknowledgement:** This work was supported by P30 CA006973 and NIH P50 CA103175.

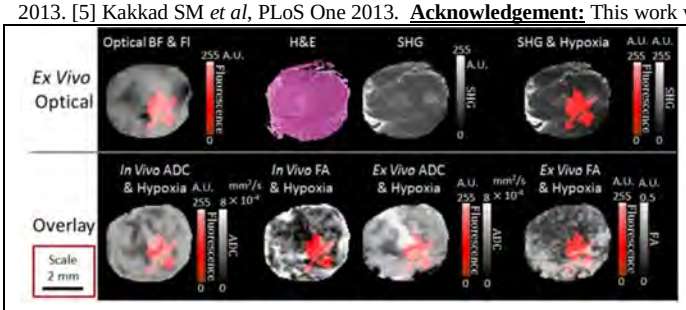

Figure 1: Top panel (left to right) - optical image with hypoxic regions in red, corresponding H&E section, SHG image showing the Col1 fiber distribution, and overlay of hypoxic regions with the Col1 fiber distribution. Lower panel (left to right) - overlay of hypoxia in red with *in vivo* ADC map, *in vivo* FA map, *ex vivo* ADC, and *ex vivo* FA maps.

	Normoxia	Hypoxia	Necrosis
ADC	Intermediate	Low	High
FA	High	Intermediate	Low
SHG	High	Low	N/A

Figure 3: Summary of the ADC and FA distributions for normoxic, hypoxic and necrotic regions. High Col1 fiber density had higher ADC and FA values as compared to the low Col1 fiber density regions.

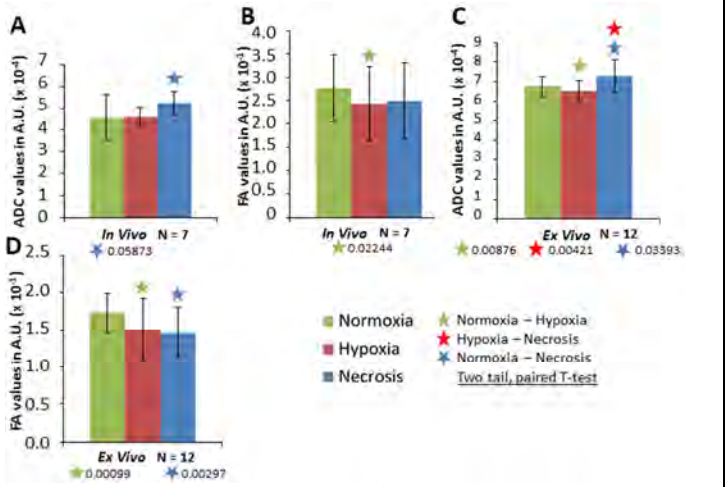

Figure 2: (A&C) Necrotic regions had significantly higher ADC values in both *in vivo* (p-value=0.06) and *ex* vivo (p-value=0.02) data as compared to normoxic and hypoxic regions (p-value=0.01). (B&C) Significantly higher FA values were observed in normoxic regions as compared to hypoxic and necrotic regions *in vivo* (p-value=0.02) and *ex vivo* (p-value=0.00) data.