Collagen fibers mediate water diffusion and anisotropy in breast tumors

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Introduction: Collagen 1 (Col1) fibers play an important role in molecular transport and cancer cell dissemination. Our goal in this study was to understand the influence of Col1 fibers on water diffusion, and to examine the potential of using noninvasive diffusion tensor imaging (DTI) to detect Col1 fibers in breast lesions. We previously observed in genetically engineered human MDA-MB-231 breast cancer xenograft tumors that fluorescence under hypoxia, relatively low amounts of collagen 1 fibers in fluorescent hypoxic regions [1]. This model was used to further investigate the relationship between Col1 fibers detected by second harmonic generation (SHG) microscopy, water diffusion and anisotropy, and hypoxia. We observed that water diffusion followed the Col1 fiber distribution, and that reduced Col1 fibers in hypoxic regions significantly decreased apparent diffusion coefficient (ADC) and fractional anisotropy (FA) values. We performed in vivo DTI measurements to confirm that DTI patterns in tumors observed in vivo spatially co-localized with DTI patterns observed ex vivo in the same tumors. These studies identify the potential use of noninvasive DTI as a surrogate marker to detect Col1 fiber density and further confirm the importance of Col1 fibers in molecular transport through the extracellular matrix (ECM).

Methods: Female severe combined immunodeficient mice were inoculated in the mammary fat pad with 2x10⁶ MDA-MB-231 cells stably expressing red florescence protein (RFP) under the control of a hypoxia response element (HRE) [2]. Once the tumor volumes were approximately 300-400 mm³, a horizontal 11.7T Bruker system (Bruker Corp.) was used to acquire in vivo DTI with a 10 mm diameter solenoid coil. Mice were anesthetized with isoflurane and the respiration was monitored. DTI was acquired with 5 non-diffusion weighted and 30 diffusion directions, (b-value = 1500 s/mm², resolution = 105 x 105 μm² x 20 z-slices). Following in vivo DTI, the tumor was excised and fixed in 4% paraformaldehyde. A vertical 11.7 Tesla spectrometer (Bruker Corp.) was used to acquire ex vivo DTI. DTI of ex vivo samples was performed in three dimensions (3D) with two non-diffusion weighted images and eight diffusion-weighted images (b=1500 s/mm², resolution 60 x 60 x 60 μm³). ADC and FA maps were calculated for both in vivo and ex vivo DTI. Following DTI acquisition, the tumor was sectioned at 1 mm slice thickness for optical imaging. Hypoxic regions were visualized by fluorescent microscopy using a 1x objective attached to a Nikon microscope. Tiled scan Col1 fibers distributions in 3D were written in MATLAB (Mathworks Inc.) to quantify for inter-fiber distance and percent fiber volume [1]. H&E sections were co-registered to corresponding DTI images using affine transformation to detect necrotic regions, which were eliminated from quantification analysis.

Results and Discussion: Heterogeneous ADC and FA value distributions were identified in the DTI data acquired from both in vivo and ex vivo DTI (Figures 1A-D). The red fluorescent hypoxic regions were identified from the optical images (Figure 1F). Overall, hypoxic regions had lower Col1 fibers, and water diffusion (ADC) and diffusion anisotropy (FA) values than normoxic regions (Figure 2). A strong correlation was observed between spatially co-localized in vivo and ex vivo ADC maps as shown in Figure 3A and a trend towards good correlation between in vivo and ex vivo FA maps as shown in Figure 3B. Here we have shown that Col1 fibers can enhance water diffusion and increase diffusion anisotropy. Noninvasive DTI may be used as a surrogate marker to assess Col1 fiber density in breast cancers, which is important because high Col1 fiber density is associated with mammary tumor initiation, progression and metastasis [3]. The low ADC and FA observed in low Col1 fiber containing hypoxic regions indicate a functional role of these fibers in molecular transport. These results strongly support investigating the use of ADC and FA to noninvasively image Col1 fiber density as well as hypoxia.

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Figure 1: Apparent diffusion co-efficient (ADC) and fractional anisotropy (FA) map from in vivo DTI data (A-B); corresponding sections of ADC and FA map from ex vivo DTI data (C-D); corresponding H&E section (E); and corresponding registered optical images, hypoxic regions in red overlaid with SHG image showing Col1 fiber distribution (F).

Figure 2: (A) Percent fiber volume was significantly lower in hypoxic regions as compared to normoxic regions (p-value = 0.0383, N = 3). Significantly lower (B) ADC and (C) FA values were observed in hypoxic regions as compared to normoxic regions (p-value = 0.0592 and p-value = 0.0308, respectively, N = 5).

Figure 3: Correlation analysis between in vivo and ex vivo ADC values (A), showing a good correlation with co-relation coefficient of 0.80 and FA values (B), showing a trend towards good correlation with co-relation coefficient of 0.56.