Mapping collagen 1 fiber architecture to diffusion tensor imaging in human breast tumor specimens

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Introduction: Collagen 1 (Col1) fibers are integral part of the tumor extracellular matrix (ECM) and play and important role in macromolecular transport and cancer cell dissemination. High Col1 fiber density is a hallmark of malignant breast cancer and is shown to cause mammary tumor initiation, progression, and metastasis [1]. Our aim was to determine the influence of the Col1 fiber architecture in human breast cancer on water molecular diffusion and evaluate the potential of diffusion MRI in studying Col1 fibers in cancer. We have developed an automated analysis technique to evaluate and segment the complex Col1 fiber patterns using Haralick texture feature analysis. Here for the first time we found that high Col1 fiber density regions had relatively higher apparent diffusion coefficient (ADC) and fractional anisotropy (FA) values as compared to low Col1 fiber density regions. However, in tightly packed high Col1 fiber density regions, water diffusion was restricted, and the ADC and FA values were reduced. We were able to map Col1 fibers to water diffusion and these results suggest that diffusion MRI may be used to assess Col1 fiber architecture and density in breast cancer.

Methods: Fixed infiltrating ductal carcinoma (IDC) samples (stage IIb, grade 3, ER+, PR+, HEr2+) were spatially marked and placed in a 10 mm NMR tube immersed in Fomblin perfluoro polyether solution (Solvay Solexis). Data were acquired on an 11.7 Tesla spectrometer (Bruker Biospin) using a 10-mm diameter volume coil. High-resolution T2- and T2*-weighted MRI, and DTI of the sample was performed in three dimensions (3D) with two non-diffusion weighted images and six diffusion-weighted images (b=1500 s/mm2) (resolution 60 x 60 x 60 μm^3). Apparent diffusion coefficient (ADC) and fractional anisotropy (FA) maps were calculated. The sample was then frozen and cryo-sectioned at 100 µm. A Zeiss LSM 710 NLO multiphoton microscope was used to image Col1 fibers by second harmonic generation (SHG) microscopy with an incident laser line of 880 nm, detected at 410-470 nm. Tile scans of 22 by 20 tiles (1 tile FOV = 339.84 µm x 339.84 µm) were acquired to cover the entire breast tumor section using a 25x lens. The analysis was done in MATLAB (Mathworks Inc.). Two major structural characteristics of the Col1 fiber network in the ECM were computed using image processing techniques: 1) spatial characteristics using Euclidean distance maps to collectively represent the sparseness and density of the distribution [2]; 2) Col1 fiber distribution pattern characteristics were computed by textural analysis method using a co-occurrence matrix approach proposed by Haralick et al [3]. The Haralick's co-occurrence matrix extracts fibers and finds spatial inter-relations of the fibers that vary across the tissue from straight/aligned fibers to curly/crisscross fibers described by 13 Haralick features [3]. We segmented the fibers by fuzzy C-mean clustering of the Haralick texture features, and color-coded the 3D projected image to represent the 3D fiber information in 2D (Fig. 1b & d). The Col1 fiber images were registered to the DTI images and 5 regions of interests (ROI) were picked for each, in dense fibers region, mixed distribution and no or few fiber distribution region. Average ADC and FA values were calculated for these regions. Additionally the texture analysis parameters, and fiber volume and inter-fiber distances were calculated for these ROIs. A two tail t-test was performed and p-value<0.05 was considered significant.

Results and Discussions: The Haralick feature based fiber segmentation gave a unique color code for densely packed, mixed distribution and sparsely distributed Col1 fibers (Fig. 1b &d). Water diffusion seemed to follow the Col1 fiber distribution (Fig. 1). Regions with higher Col1 density had higher ADC and FA values than regions with few or no fibers. However, mixed fiber distribution regions had ADC and FA values higher than the densely packed fibers (Fig. 1e). This is because the mixed fibers had a higher inter-fiber distance and were more sparsely distributed than the densely packed fibers allowing water to diffuse between the fibers more easily. While in regions with no or very few Col1 fibers the ADC and FA values were significantly lower (p-value<0.01) than regions with Col1 fiber present (Fig. 1e). Noninvasive DTI may be used 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 [1].

Reference: [1] Provenzano P. P. et al., BMC Med 2008; [2] Kakkad et al., Neoplasia, 2010; [3] Haralick et al., IEEE Transactions on systems, man and cybernetics, 1973. **Acknowledgement:** This work was supported by NIH P50 CA103175 and P30 CA006973.

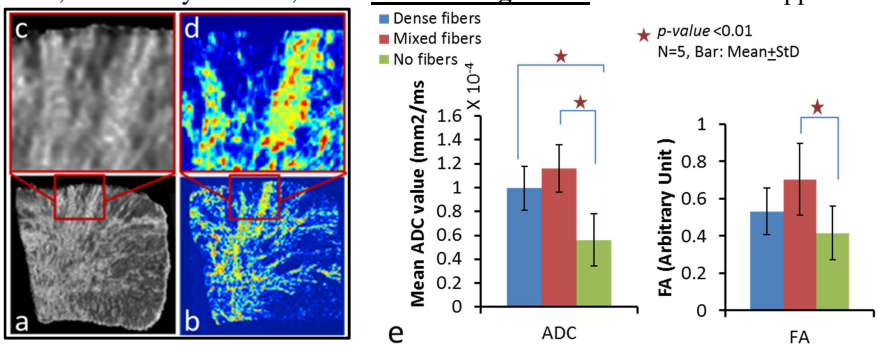

Figure1: (a) ADC map; (b) corresponding SHG tile-scan of Col1 fibers map, texture analysis color coded for high, mixed & low dense fiber distribution; (c) enlarged ADC box from (a); (d) enlarged SHG color coded Col1 box from (b). The dark red color represents curly fibers and the light blue color represents straight and more sparely distributed fibers. (e) Graphs for apparent diffusion coefficient (ADC) and fractional anisotropy (FA) for densely, mixed and very sparsely distributed fiber ROIs, N=5.