Visualizing Collagen I fiber architecture in human breast tumor specimens using Diffusion Tensor Imaging

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Introduction

Malignant breast cancers are characterized by significantly higher collagen I (Col1) fiber density and an altered Col1 fiber architecture [1]. High mammary Col1 fiber density was shown to cause mammary tumor initiation, progression, and metastasis [1]. Our goal is 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 performed ultra-high-resolution diffusion tensor imaging (DTI) of a human breast cancer specimen *ex vivo*, which was subsequently sectioned and imaged by second harmonic generation (SHG) microscopy to detect intrinsic signal from Col1 fibers. We found, for the first time, that high Col1 fiber density correlated with the apparent diffusion coefficient (ADC), suggesting that Col1 fibers may enhance water diffusion by facilitating molecular transport along Col1 fibers in breast cancers. In close proximity to dense Col1 fibers, we observed relatively high diffusion anisotropy probably due to the presence of Col1 fibers. These results suggest that diffusion MRI may be used to assess Col1 fiber architecture and density in breast lesions, which will be significantly 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 [1].

Methods

An infiltrating ductal carcinoma sample (stage IIb, grade 3), which was positive for estrogen receptor, progesterone receptor, and Her2 was fixed for 24 hours in 4% paraformaldehyde, and washed with phosphate buffered saline for 72 hours. This sample was spatially marked and placed in a 10-mm NMR tube immersed in Fomblin perfluoro polyether solution (Solvay Solexis). MRI was performed 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 this sample were performed in three dimensions (3D) with an isotropic resolution of 60 x 60 x 60 μm^3. For DTI, two non-diffusion weighted images and six diffusion-weighted images ($b=1500$ s/mm^2) were acquired. From these images, the average diffusion-weighted (aDW) images and maps of ADC and fractional anisotropy (FA) were calculated. Following MRI, the sample was frozen and cryo-sectioned along the z-axis representing the B0 field direction of MR acquisition at 100-µm thickness and thaw-mounted on microscope slides. We used a Zeiss LSM 710 NLO multiphoton microscope to image Col1 fibers by SHG microscopy with an incident laser line of 880 nm, detected at 410-470 nm. To cover the entire breast tumor section in x and y with the much smaller field of view (FOV) of the microscope's 25x lens (339.84 µm x 339.84 µm), we performed a tile scan of 22 by 20 FOVs. The SHG microscopic tile-scan was overlaid with the corresponding z-plane image of the DWI and DTI 3D data set.

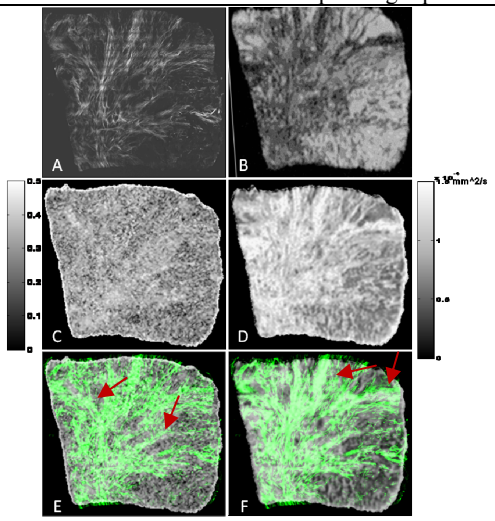

Figure 1: (**A**) SHG tile-scan image of Col1 fibers, corresponding **(B)** aDW image, **(C)** FA image, and **(D)** ADC map. **(E, F)** show registered overlays of **(E)** FA (gray) and **(F)** ADC (gray) with the corresponding SHG Col1 fiber image (green). Arrows point out areas in which Col1 fiber density correlates with FA and ADC.

Results

We observed a positive correlation of SHG-detected Col1 fibers with the corresponding ADC map measured by DTI in a human invasive ductal carcinoma as shown in Figure 1. The merged image in Figure 1F clearly demonstrates that dense Col1 fibers were detected in regions of high ADC (see arrows), while regions of low ADC values contained no Col1 fibers. The FA image corresponding to the SHG tile-scanned Col1 fiber image (Figures C, E) shows that a high degree of fractional anisotropy was detected in regions of high Col1 fiber density, whereas regions without Col1 fibers displayed relatively lower diffusion anisotropy (see arrows).

Discussion

We were able for the first time to image co-registered SHG-detected Col1 fibers and ultra-high-resolution DTI to investigate the influence of Col1 fibers on the properties of water diffusion. We used ultra-high-resolution DTI to examine fiber architecture at a microscopic scale to reveal its complexity. Our finding that Col1 fiber density positively correlated with ADC values suggest that Col1 fibers might enhance water diffusion in malignant breast cancers that are characterized by high Col1 fiber content. In areas of dense Col1 fibers, we observed an increased fractional anisotropy as detected by DTI. This is in good agreement with a recent DTI study of human articular cartilage, in which the directionality of water diffusion was consistent with the zonal distribution of Col1 fiber orientation [2]. Recent clinical *in vivo* studies demonstrated that the ADC in breast cancers was significantly lower than that of benign breast lesions and normal breast tissue [3, 4], accompanied by decreased fractional anisotropy in cancer *versus* normal [4]. In comparison, our study was performed at much higher spatial resolution (0.06 mm *vs.* 2 mm) in *ex vivo* fixed tissue, which may explain the differences in findings. We are currently investigating normal breast tissue with our combined high-resolution *ex vivo* SHG-Col1 fiber and DTI approach. In conclusion, we have shown that dense Col1 fibers in breast cancers can enhance water diffusion and increase diffusion anisotropy. Noninvasive DWI and/or 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 [1].

References

[1] Provenzano P. P. et al., BMC Med 6, 11 (2008); [2] Xiang D. et al., Magn Reson Imaging 25, 168-171 (2007); [3] Khouli R. H. et al., Radiology 256, 64-73 (2010); [4] Savannah C. P. et al., JMRI 31, 339-347 (2010).

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