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Collagen fibers mediate MRI-detected water diffusion and anisotropy in breast cancers

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Abstract

Collagen 1 (Col1) fibers play an important role in tumor interstitial macromolecular transport and cancer cell dissemination. Our goal was to understand the influence of Col1 fibers on water diffusion, and to examine the potential of using noninvasive diffusion tensor imaging (DTI) to indirectly detect Col1 fibers in breast lesions. We previously observed, in human MDA-MB-231 breast cancer xenografts engineered to fluoresce under hypoxia, relatively low amounts of Col1 fibers in fluorescent hypoxic regions. These xenograft tumors together with human breast cancer samples were used here to investigate the relationship between Col1 fibers, water diffusion and anisotropy, and hypoxia. Hypoxic low Col1 fiber containing regions showed decreased apparent diffusion coefficient (ADC) and fractional anisotropy (FA) compared to normoxic high Col1 fiber containing regions. Necrotic high Col1 fiber containing regions showed increased ADC with decreased FA values compared to normoxic viable high Col1 fiber regions that had increased ADC with increased FA values. A good agreement of ADC and FA patterns was observed between *in vivo* and *ex vivo* images. In human breast cancer specimens, ADC and FA decreased in low Col1 containing regions. Our data suggest that a decrease in ADC and FA values observed within a lesion could predict hypoxia, and a pattern of high ADC with low FA values could predict necrosis. Collectively the data identify the role of Col1 fibers in directed water movement and support expanding the evaluation of DTI parameters as surrogates for Col1 fiber patterns associated with specific tumor microenvironments as companion diagnostics and for staging.

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Introduction

Collagen 1 (Col1) fibers are a major structural component of the extracellular matrix (ECM) of tumors [1]. Increased mammary Col1 fiber density was shown to cause mammary tumor initiation, progression, and metastasis [2]. Col1 fiber topography including fiber diameter [3], directionality [4] and alignment [5] play an

Abbreviations: 2D, two-dimensional; 3D, three-dimensional; aDW, average diffusion weighted; ADC, apparent diffusion coefficient; Col1, collagen 1; DTI, diffusion tensor imaging; ECM, extracellular matrix; ER, estrogen receptor; FA, fractional anisotropy; FOV, field of view; H&E, hematoxylin and eosin; HER-2, human epidermal growth factor receptor 2; HIF-1, hypoxia inducible factor; HRE, hypoxia response element; IDC, invasive ductal carcinoma; LN, lymph node; MRI, magnetic resonance imaging; PFA, paraformaldehyde; PR, progesterone receptor; RFP, red fluorescence protein; SCID, severe combined immunodeficient; SHG, second harmonic generation; VEGF-A, vascular endothelial growth factor A

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important role in cancer motility and invasiveness. Cancer cells can travel along aligned Col1 fibers [6,7]. Tumor associated Col1 fiber alignment is a potential prognostic signature for survival in breast cancer patients [8]. Col1 fibers can be detected with second harmonic generation (SHG) microscopy [1] that detects an intrinsic signal derived from the noncentrosymmetric molecular structure of Col1 fibers [1,9]. Because of limited depth penetration, unless the tissue is superficial, exposed for *in vivo* imaging, or biopsied for *ex vivo* imaging, SHG microscopy cannot be used as a noninvasive imaging modality.

In the brain, water molecules predominantly diffuse along neuronal fibers as diffusion across fibers is restricted by axonal membranes and, in the case of myelinated axons, myelin sheaths. As a result diffusion imaging parameters, *e.g.* apparent diffusion coefficient (ADC) and fractional anisotropy (FA), have been used to visualize fiber tracts and quantitatively measure white matter integrity noninvasively [10–13]. Col1 fibers in the tumor ECM may mediate, in a similar fashion, water diffusion. Diffusion tensor imaging (DTI) detected Col1 architecture, as validated by SHG microscopy, in the carotid artery [14], identifying the potential use of DTI as a noninvasive imaging technique to detect Col1 fibers [14]. Here we investigated the association between diffusion MRI parameters and breast cancer Col1 fiber distribution, to determine if diffusion MRI can provide noninvasive imaging indices of Col1 fibers to use as surrogate markers of aggressiveness.

We determined the correlation between Col1 fibers and water diffusion in MDA-MB-231 human breast cancer xenografts engineered to express red fluorescent protein (RFP) under hypoxia [15], since we previously observed that hypoxic regions contained significantly fewer Col1 fibers [15]. This provided a useful model to easily identify low Col1 fiber regions to relate to the two DTI parameters, apparent diffusion coefficient (ADC) and fractional anisotropy (FA). Additional *ex vivo* studies were performed with human specimens to confirm the observations made with xenografts. Our data identified a close association between Col1 fiber density and DTI parameters in the *in vivo* and *ex vivo* studies supporting further evaluation of ADC and FA as noninvasive indices of Col1 fibers in tumors, and highlighting the importance of Col1 fibers in molecular transport through the ECM.

Methods

Breast Cancer Xenografts

Orthotopically implanted MDA-MB-231 tumors derived from cells containing the hypoxia response element (HRE) of the human vascular endothelial growth factor A (VEGF-A) gene ligated to the cDNA of tdTomato RFP (MDA-MB-231-HRE-TdTomato) were used to identify hypoxia [15,16]. *In vivo–ex vivo* DTI studies were performed on six tumors with an additional five used for *ex vivo* studies alone. An overview of the experimental design is presented as a schematic in Figure 1.

Clinical Specimens

Studies with clinical specimens were performed to determine if the observations made with xenografts were replicated in human specimens. DTI and SHG microscopy were performed on one stage IIB and two stage IIIA invasive ductal carcinoma (IDC) with an approximate size of 10 × 15 mm^2, obtained from Integrated Laboratory Services–Biotech (Chestertown, MD, USA). All tumors were grade 3, estrogen receptor positive (ER+), progesterone receptor

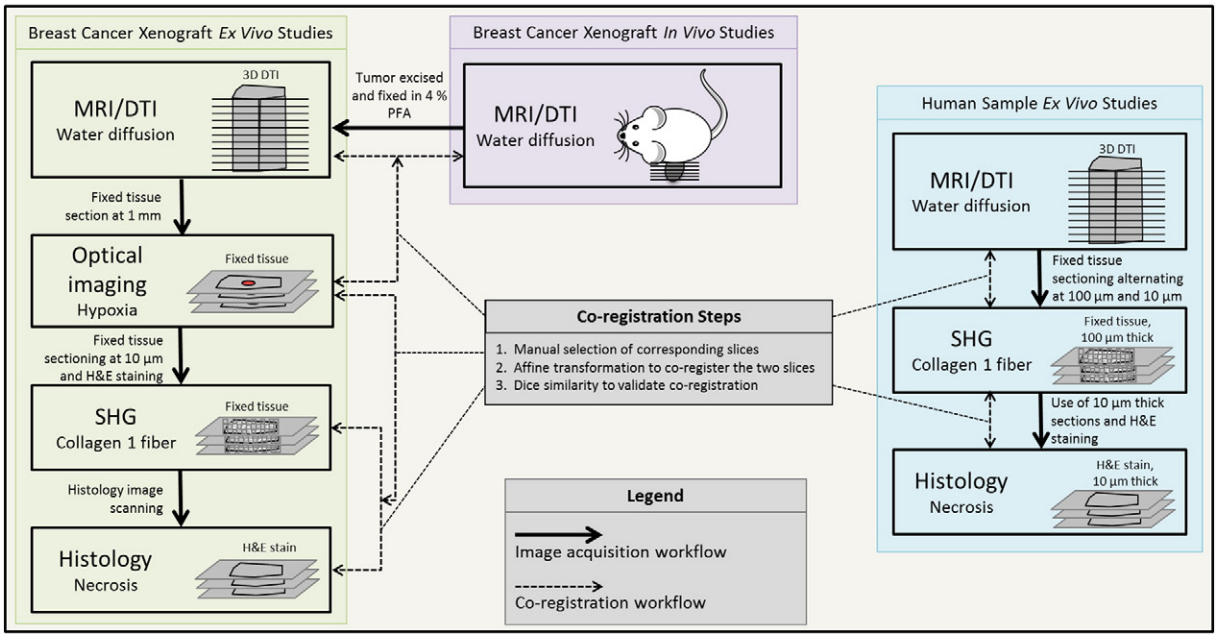

Figure 1. Schematic outline of the workflow. The green box summarizes the *ex vivo* study workflow of breast cancer xenografts. The purple block summarizes the *in vivo* study workflow of breast cancer xenografts. DTI acquisitions were done *in vivo*. Excised tumors were put through the experimental and analytical steps as described in the workflow. Co-registered *in vivo* and *ex vivo* images were used to calculate the correlation between *in vivo* and *ex vivo* measurements for ADC and FA values in hypoxic, normoxic and necrotic regions. The blue box summarizes *ex vivo* study workflow of human breast cancer specimens. Thick arrows indicate the image acquisition workflow, while dashed arrows represent the quantification and analysis workflow.