Multi-scale Imaging of Angiogenesis in a Breast Cancer Model

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Introduction: Angiogenesis in breast cancer helps meet the metabolic demand of the tumor and favors dissemination and metastasis [1]. Poor prognosis correlates with increased vascular density and production of proangiogenic factors [2], some of which have been used as therapeutic targets [3]. These developments have heralded a need for new methods to characterize angiogenesis in pre-clinical models of breast cancer. While micro-CT (μCT) is a powerful tool for dissecting the tumor microvasculature, *in vivo* MRI with its sub-millimeter resolution is useful for obtaining vascular data (e.g. blood volume and vessel size index) at the "systemic" scale. Here we demonstrate the feasibility of imaging breast cancer angiogenesis using a "multi-scale" imaging approach, wherein we bridge the resolution gap between μCT and *in vivo* MRI using MR microscopy (μMRI).

Methods: "Multi-scale" images of MDA-MB-231 breast tumors were acquired using three different methods: (i) 10 breast tumor bearing mice were imaged *in vivo* on a 9.4T horizontal bore scanner at 100μm in-plane resolution and 1mm slice thickness using 2D T2-weighted (T2w) RARE and 2D T2*w multi-echo gradient echo (MEGE) sequences. Both scans were performed before and after intravenous Feridex® (Bayer Healthcare Pharmaceuticals) injection. (ii) Following *in vivo* MRI, mice were perfusion-fixed and perfused with Microfil® (FlowTech Inc., MA), a radio-opaque silicone compound. Tumors were excised, fixed in zinc formalin and imaged *ex vivo* on a 400MHz spectrometer at 40μm isotropic resolution using a 3D T2*w MEGE sequence. (iii) 3D μCT data were acquired at 8μm isotropic resolution. From the *in vivo* data, ΔR2 and ΔR2* blood volume maps were generated by an exponential fitting method described in [4]. The 3D tumor vasculature was extracted from μMRI and μCT data by applying a Hessian-based filter [5] followed by binarization. For the μMRI k-means clustering was also used to eliminate necrotic areas. 3D μMRI data were co-registered to the *in vivo* MRI data using images from the first echo time (TE), followed by co-registration of the μCT data to the μMRI data using the segmented vasculature. The three datasets were aligned via manual rigid-body registration. Fractional blood volume (FV) maps were computed from high-resolution μMRI and μCT vasculature by using the *in vivo* MRI spatial grid as a template and calculating the fractional occupancy of vessels for each *in vivo* voxel [4].

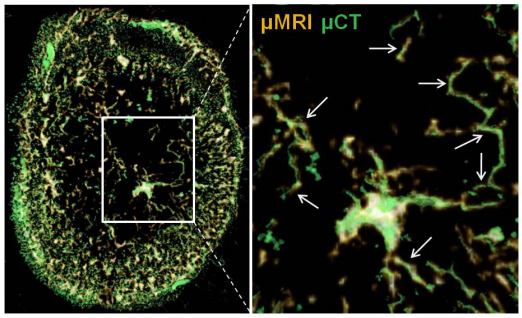

Fig. 1: Thin slice of co-registered μMRI (yellow) and μCT (green) blood vessels (left). Enlarged cut-out displaying excellently co-registered blood vessels (right).

Results: The co-registration of the 3D *ex vivo* μMRI and μCT data resulted in a remarkable overlap between the majority of tumor blood vessels, while the smallest vessels were only visible in the μCT data (**Fig.1**). The co-registered *in vivo* ΔR2*(global blood volume)- and *ex vivo* FV-maps showed excellent correspondence (**Fig.2**). Region of interest analysis demonstrated significantly ($p<0.001$, KS-test) lower FV in the tumor core compared to the rim at all three spatial scales. This was accompanied by a greater number of necrotic regions in the center relative to the rim, as identified on the T2*w μMRI images. The FV from the μMRI data was elevated relative to that obtained from the μCT data.

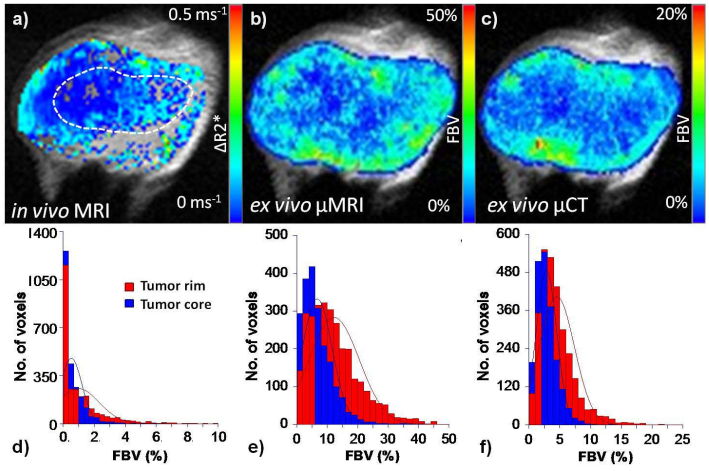

Fig. 2: Co-registered maps of: (a) *in vivo* ΔR2*, (b) μMRI and (c) μCT fractional blood volume overlaid on an *in vivo* T2w image. (d-f) Distributions of ΔR2* and FV demonstrating elevated values for the angiogenic tumor rim versus the necrotic core.

Discussion: For the first time we have generated co-registered blood volume maps with complementary contrast mechanisms to reflect the angiogenic status of a breast cancer model at multiple spatial scales. "Intermediate" scale 3D μMRI of the tumor vasculature is a novel high-resolution method for quantifying angiogenic changes, and enables validation of the *in vivo* MRI blood volume measurements against μCT by facilitating accurate co-registration. As the resolution of μMRI is five times lower than that of μCT, partial volume effects lead to higher FV estimates in the former. The lower FV in the tumor center relative to the rim was due to the poor vascularization of the necrotic regions in the center compared to the well-vascularized rim.

Conclusion: Multi-scale imaging of the angiogenic phenotype facilitates validation of *in vivo* markers of tumor angiogenesis, and helps establish physiologically accurate models of angiogenesis and image contrast. Multi-resolution imaging paves the way for an integrated platform to study the "system biology" of breast cancer.

References: **1.** Saaristo et al., *Oncogene*, 19(53):6122-9, 2000. **2.** Weidner et al., *J Natl Cancer Inst*., 16;84(24):1875-87,1992. **3.** Bossung et al., *Curr Opin Obstet Gynecol.* 22(1):79-86, 2010. **4.** Pathak et al., *Mag Reson Med.*, 46(4):735-47, 2001. **5.** Sato et al., *Med. Image Anal.*, 2(2):143-168, 1998.

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