Characterizing Hypoxia-induced Alterations in ECM Integrity of Solid Tumors In Vivo Using MRI and Fluorescent Microscopy

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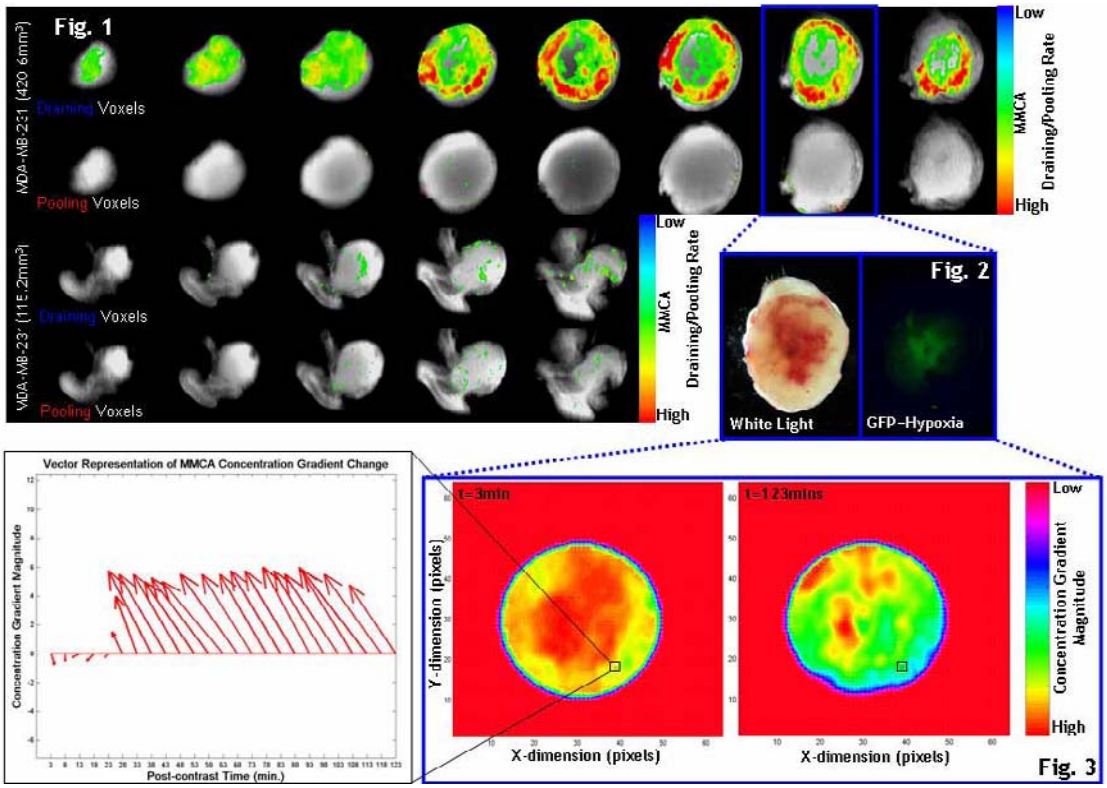
INTRODUCTION: Tumor microenvironmental factors are critical determinants in the phenotypic traits of cancer. Of these, hypoxia is known to induce drug/radiation resistance, and select for a more aggressive phenotype. However, the relationship between hypoxia, the resultant ECM remodeling and macromolecular transport *in vivo* remains largely unexplored. Hypoxia is known to induce collagen deposition [1], and induce upregulation of various matrix metalloproteinases (MMPs) [2] that remodel the ECM and affect the movement of macromolecules. Recently we developed a noninvasive method for characterizing transport of macromolecules through the ECM of tumors *in vivo* using MRI [3]. Here for the first time we have related the macromolecular movement through the ECM to tumor hypoxia, using optical molecular imaging of tumor hypoxia in conjunction with novel MRI analyses that further extends our earlier technique.

METHODS: MRI of two anesthetized MDA-MB-231-GFP-HRE tumor-bearing mice, selected for differences in tumor size (420.6mm^3 vs. 115.2mm^3) was conducted. In these tumor cells, expression of green fluorescent protein (GFP) was placed under the control of a hypoxia response element (HRE) which contains binding sites for hypoxia inducible factor-1 (HIF-1), enabling us to detect HIF-1 activity. As HIF-1 expression is tightly regulated by cellular oxygen concentrations, hypoxia resulted in GFP expression that was imaged in freshly excised tumor sections, post-MRI. First, multi-slice T1 relaxation rates of the tumor were obtained by saturation recovery combined with SNAPSHOT FLASH. Five to eight 1mm slices were acquired (256×256μm^2 resolution) for three relaxation delays (100, 500 and 1000ms). Images were obtained before i.v. administration of 0.2ml of 60mg/ml albumin Gd-DTPA contrast agent (CA) and repeated every 5 min, starting at 3 min post-injection, up to 140 min post contrast since the transport of macromolecules within the ECM is slow. After each study, mice were sacrificed and blood T1's determined from tail vein samples. Parameters describing ECM transport such as draining and pooling rates within tumor voxels were calculated as described in [3]. In addition, the time-evolution of the vector gradient of the contrast agent concentration within each voxel was computed for each experimental time point using MATLAB®: concentration-time ($C(t)=\Delta R1_{Tissue}(t)/\Delta R1_{Blood}(t)$) images were converted to 8-bit grayscale TIFs, sub-sampled to expedite computation, masked with a circular mask to eliminate spurious gradients at the image edges, and the 2D vector gradient computed for each time point ($\nabla C = \frac{\partial C}{\partial x}\hat{i} + \frac{\partial C}{\partial x}\hat{j}$). The gradient magnitude was displayed as a colormap for each time point.



RESULTS: The upper panel of **Fig. 1** illustrates the higher rate and larger extent of CA drain within the ECM of the larger tumor compared to the smaller tumor (**Fig. 1** lower panel). In addition, the larger of the two tumors exhibited hypoxic regions, evident from the GFP image (**Fig. 2**). The closest matching MRI slice is shown within the box in **Fig. 2**. The first panel in **Fig. 3** illustrates the magnitude of the concentration gradient corresponding to t=3min and t=123 min post-contrast, respectively (for the slice within box). One can clearly distinguish spatial changes in this gradient over time. Additionally, the final panel of **Fig. 3** illustrates the time-evolution of the magnitude and direction of the CA gradient for an ECM voxel.

DICUSSIONS: The larger of the two tumors not only exhibited large central area of necrosis, but consistent with the possibility of ECM remodeling in response to hypoxia-induced upregulation of cytokines such as MMPs, CA drainage rates around hypoxic regions (box in **Fig. 1**) were much higher than in the smaller tumor. Additionally, *vector* gradient maps enable not only the visualization of such ECM events in relation to microenvironmental cues such as hypoxia, but also allow us to track the directionality of extravascular transport events. Currently, efforts are under way to extend this analysis to 3D with concurrent registration of the optical and MR images using an affine transformation. While these preliminary data demonstrate the feasibility of such an approach, studies with a larger cohort of animals are currently underway. appreciate their spatio-temporal evolution

CONCLUSIONS: Dual modality imaging approaches such as this one allow us to harness the advantages of each modality. Fluorescent microscopy enables molecular imaging of hypoxia while MRI enables an *in vivo* assessment of ECM integrity and transport. The two in conjunction help us better understand the interplay of microenvironmental signals, such as cellular hypoxia and ECM remodeling and their role in tumor progression as well as therapy.

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