A New Method for Phenotyping the Brain Tumor Microenvironment Using MR Microscopy

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INTRODUCTION: Brain tumors have highly abnormal vascular morphology, a critical determinant of their pathophysiology, therapeutic efficacy and image contrast in MRI [1]. The vascular architecture of high-grade gliomas is also strongly dependent on tumor size, location within the tumor and local angiogenic activity [2]. The key role of tumor angiogenesis, along with the development of mouse models of anti-angiogenic therapy, has created a crucial need to characterize the murine vascular phenotype [3]. Here we describe a new method using high-resolution 3D magnetic resonance microscopy (μMRI) for characterizing changes in the vascular phenotype of a brain tumor model with tumor progression.

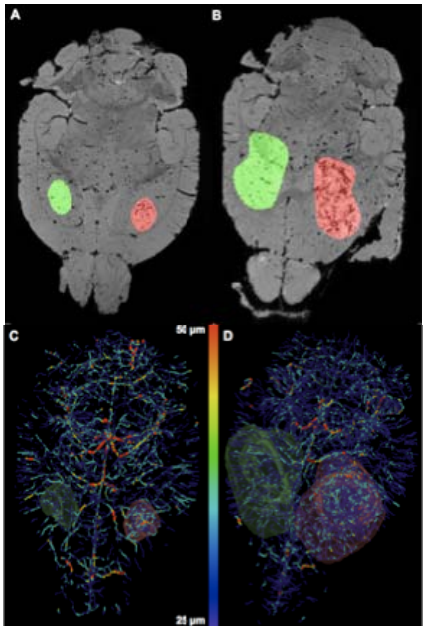

Fig. 1. Axial slices from the first-echo of the GE μMRI of a D12 (A) and D17 (B) tumor-bearing brain. Tumor and CL ROIs are overlaid in red and green, respectively. (C, D) 3D vasculature color-coded by vessel radius. ROIs defined in (A-B) are also rendered in 3D.

METHODS: Ten 9L brain tumor bearing mice were perfusion fixed then subsequently perfused with a silicone compound called Microfil® (FlowTech Inc., MA) using a method we previously developed [4]. Five animals were sacrificed on post-inoculation day 12 (D12) and the other five on post-inoculation day 17 (D17). Post-fixation, brains were extracted from their skulls and imaged on a 400MHz spectrometer at ~60μm^3 resolution using the following 3D sequences: T2*-weighted (T2*w) multi-echo gradient echo (GE), T2w RARE and diffusion-weighted RARE (dwRARE). The 3D vasculature was extracted from the GE images using a "tubeness" filter based on the Hessian eigenvalues [5]. This was followed by binarization of the vascular structure (**Fig. 1**), from which vessel branch length, vessel radius, microvessel density (MVD), length per unit volume (L_V), fractional blood volume (FV) and tortuosity were computed for manually drawn tumor and analogous contralateral (CL) regions of interest (ROI). Voxel-wise ADC and FA maps were computed from dwRARE images for the same ROIs. The spatial distributions of these parameters within the tumors were also analyzed by defining rim, intermediate and core zones by performing successive 3D morphological erosions on the tumor ROIs.

RESULTS: With the exception of D12 L_V and D17 tortuosity, every μMRI parameter was significantly ($p<0.05$) different in tumor ROIs than CL ROIs. D17 L_V and D12 tortuosity were significantly greater in tumors. For both D12 and D17 groups, vessel radius, MVD, FV, ADC and FA were elevated in tumor with respect to CL; branch length displayed the opposite relationship. MVD, L_V and FV were also significantly higher in D12 tumors than D17 tumors. However, these parameters were highest in D12 tumor cores, but lowest in D17 tumor cores. In contrast, ADC and FA did not change significantly with tumor growth, and both D12 and D17 tumors exhibited lower ADC in their rims than their interiors. **Fig. 2** shows the result of an unsupervised hierarchical clustering algorithm, which successfully classified D12 and D17 tumor and CL ROIs based on the μMRI parameters alone.

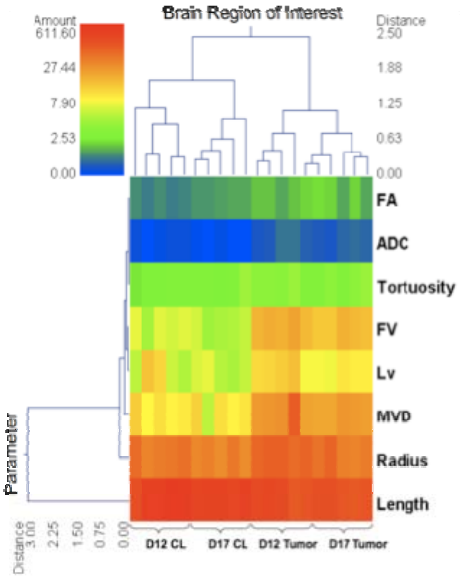

Fig. 2. Double dendrogram showing the results of the unsupervised hierarchical clustering analysis, which classified all 20 ROIs correctly by group (D12 CL, D17 CL, D12 tumor, D17 tumor).

DISCUSSION: Our data demonstrate the feasibility of using μMRI for differentiating the tumor vascular architecture from that of the CL brain and for characterizing changes in brain tumor vascular morphology with tumor progression. The shorter vessel branch length and elevated vessel radius, MVD, L_V, FV and tortuosity in tumor vs. CL ROIs are hallmarks of tumor vasculature and angiogenic activity [2, 6]. Our results also show characteristic differences in the angiogenic phenotype between D12 and D17 tumors. The spatial trends of MVD, L_V and FV in D17 tumors are consistent with previous observations that larger gliomas have well-vascularized, angiogenic peripheries and less angiogenic cores with lower vascular density [2]. In combination with diffusion-weighted MRI, we could accurately characterize the microenvironmental phenotype of the D12 and D17 ROIs.

CONCLUSION: The method presented here offers high-resolution 3D information on the vasculature of the whole mouse brain with the added advantage of soft tissue contrast and diffusion related measurements. Our data show that μMRI is an effective means of characterizing the vascular phenotype of a 9L mouse brain tumor model, as well as the microenvironmental changes that accompany brain tumor progression. Such imaging approaches can find widespread application in other pathologies involving the neurovasculature.

REFERENCES: **1.** Pathak et al., *Neuroimage*, 40(3):1130-43, 2008. **2.** Vajkoczy et al., *J Neurooncol*, 50(1-2):99-108, 2000. **3.** Raman et al., *NMR Biomed*, 20(3):186-99, 2007. **4.** Pathak et al, *Proc. ISMRM, 16th Mtg,* 2008. **5.** Sato et al. *Med Image Anal.*, 2(2):143-168, 1998. **6.** Jain et al., *Nat Rev Neurosci*, 8(8):610-22, 2007.

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