Vascular Phenotyping of Brain Tumors with MR Microscopy (μMRI)

A. P. Pathak[1], J. Zhang[1], and M. Jones[2]

[1]Russell H. Morgan Department of Radiology and Radiological Science, The Johns Hopkins University School of Medicine, Baltimore, MD, United States,
[2]Department of Neurology, The Johns Hopkins University School of Medicine, Baltimore, MD, United States

INTRODUCTION: Brain tumors remain among the most refractory of human tumors and their "angiogenic" phenotype or vascular architecture is a critical determinant of their pathophysiology, efficacy of therapy and image contrast in MRI [1]. Traditionally the angiogenic phenotype has been characterized at "cellular" spatial resolutions in terms of blood volume, vessel diameter, fractal dimension, vessel branching and connectivity using optical imaging. However, a drawback of optical imaging is that it suffers from limited coverage, and information on 3D blood vessel geometry once destroyed by sectioning requires complex reconstruction. In contrast, *in vivo* MRI with its sub-millimeter resolution has proved useful for obtaining angiogenic biomarkers such as blood volume and vessel size index at the "systemic" spatial scale [2]. Noninvasive imaging techniques that enable characterization of the angiogenic phenotype at spatial resolutions intermediate to the "cellular" and "systemic" are scarce. Here we describe a new method for characterizing the angiogenic phenotype of a brain tumor model using magnetic resonance microscopy (μMRI), which is non-destructive and preserves the 3D tissue and blood vessel architecture. When combined with different kinds of MR contrast (e.g. diffusion-tensor imaging or DTI), μMRI can provide a wealth of information on the brain tumor microenvironment that is inaccessible by other imaging methods. As μMRI generates high-resolution 3D images of the vasculature, we were able to characterize morphological differences between the angio-architecture of the contralateral brain and that of the tumor using fractal analysis.

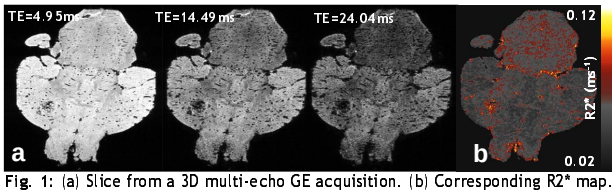


Fig. 1: (a) Slice from a 3D multi-echo GE acquisition. (b) Corresponding R2* map.

METHODS: Five 9L brain tumor (12 days post-inoculation) bearing mouse brains were perfusion fixed, followed by perfusion with a silicone rubber compound called Microfil® (FlowTech Inc., MA) according to a method developed by us earlier [3]. Post-fixation, brains were imaged on a 400MHz spectrometer with the following parameters: T2*-weighted (T2*w) multi-echo gradient echo (GE), TE=4.95/9.72/14.49/19.27/24.04/28.81ms, TR=100ms, resolution=64μm×64μm×62μm. Co-registered T2w images were also obtained TE/TR=34.5/500ms, 129μm×125μm×125μm. Matlab was used to compute 3D R2* maps on a voxelwise basis from multi-echo GE data (**Fig. 1**). Prior to fractal analysis of the vascular tree, the image volume corresponding to the 3rd TE was thresholded and filtered using a "tubeness" filter that determines how "vessel-like" each point in the image is [4]. Following re-thresholding of the "tubeness" volume, a maximum intensity projection (MIP) was generated from 5 slices of interest and the local connected fractal dimension (LCFD) computed for a tumor region of interest (TumorROI), and contralateral brain ROI (ContraROI) according to [5]. The pixel-wise LCFD map reflects the degree of space filling with a change of spatial scale for the underlying vascular structure, i.e the higher the LCFD, the more abnormal the morphology of the vasculature [5].

RESULTS: **Fig. 1** demonstrates the feasibility of obtaining whole brain vascular coverage using our Microfil® technique from which high-resolution 3D R2* maps can be constructed. **Fig. 2** shows 3D R2* maps from three 9L tumor bearing mouse brains imaged 12 days post-inoculation. At this resolution, one can clearly visualize co-option of host vessels by the tumor. Since each tumor was imaged at the same age, one can begin to appreciate the heterogeneity of their angiogenic phenotypes using this approach. **Fig. 3** illustrates results of the LCFD analysis for one brain. The LCFD maps (**Fig 3a-b**) show that the LCFD of the TumorROI (μ_{LCFD}=1.31) was higher than that of the ContraROI (μ_{LCFD} =0.98). Additionally, this difference was apparent from the LCFD distributions (**Fig 3d**) and was borne out by histology (**Fig. 3c**) wherein one can clearly see the marked difference in the angio-architecture of the tumor (T) and the normal (N) brain.

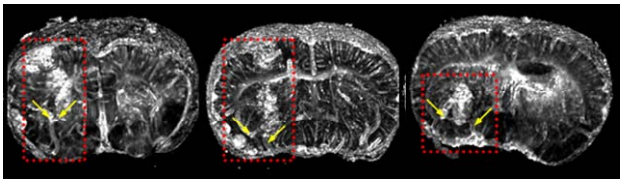
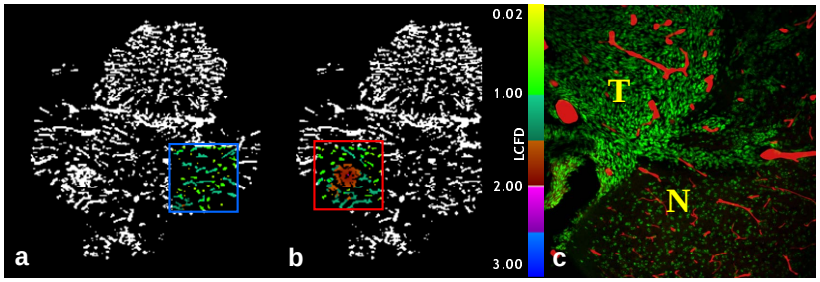


Fig. 2: 3D R2* maps from three 9L tumor bearing mouse brains. Tumors highlighted with a red rectangle in each image. At this resolution, one can clearly visualize recruitment of host vessels by the tumor (yellow arrows). Since, each tumor was imaged 12 days post-inoculation, one can appreciate the heterogeneity in their angiogenic phenotypes using this approach.



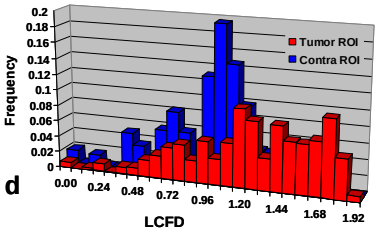


Fig. 3: (a) Binarized MIP showing the contralateral ROI (blue box) that was analyzed on a pixel-wise basis for the local connected fractal dimension (LCFD), and (b) an ROI (red box) in the tumor. Color-maps illustrating the elevated LCFD in the TumorROI. (c) Histological image of the same brain in which one can clearly see the aberrant architecture of the tumor (T) vessels in contrast to those in the normal (N) brain. (d) Distributions of the LCFDs in (a) and (b) exhibit a shift towards higher LCFDs for the TumorROI relative to the ContraROI, indicative of *abnormal* vascular architecture in the TumorROI.

DISCUSSION: The imaging method presented here exploits the susceptibility contrast induced by the presence of Microfil® to make sub-voxel sized vessels visible. High-resolution images are necessary to characterize the angiogenic phenotype, and our μMRI approach enables us to successfully characterize differences between the anomalous brain tumor vessels and those of the contralateral brain using automated fractal analysis. Unlike optical methods that only image a small tumor ROI, high-resolution μMRI enables us to quantify the heterogeneity of the angiogenic phenotype in 3D with whole brain coverage. This could prove especially important for characterizing subtle changes in the tumor angio-architecture: for example, certain vascular "normalization" therapies may produce a significant change in vessel architecture without a concomitant change in the tumor's blood volume. We are currently in the process of characterizing the evolution of the angiogenic phenotype and reorganization of the cortical white matter (using DTI) in an array of brain tumor models that includes patient-derived primary tumor xenografts.

CONCLUSIONS: Our preliminary studies demonstrate the feasibility of characterizing the angiogenic phenotype of a brain tumor model at high spatial resolution in 3D, using contrast-enhanced μMRI. Such data of the brain tumor vasculature can be used in a range of applications that includes physiologically accurate, multi-scale mathematical models of angiogenesis.

REFERERENCES: **1.** Pathak et al, *NeuroImage*, 40(3):1130-43, 2008. **2**. Pathak et al., *Angiogenesis*, Oct, 2008. **3**. Pathak et al, *Proc. ISMRM, 16th Mtg,* 2008. **4**. Sato et al. *Med Image Anal.*, 2(2):143-168, 1998. **5**. Landini et al. *Inv Ophthal Visual Science,* v36, 2749-55, 1995.

ACKNOWLEDGEMENTS: Research supported by Toshiba Medical Systems/RSNA Research Seed Grant.