

## In-vivo Biomarkers for Brain Tumor Vasculature and Cellularity Validated with Ex-vivo Tissue

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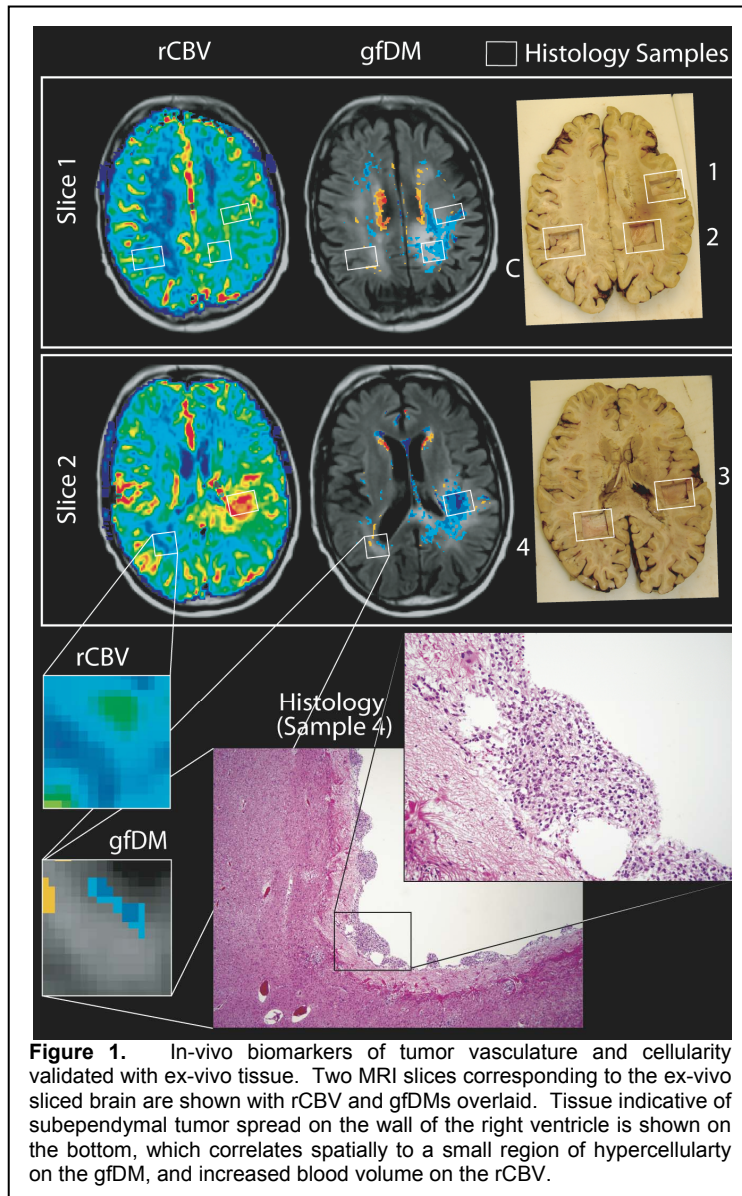
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**INTRODUCTION** The detection of invading brain tumor cells, beyond the traditional contrast-enhancing regions, continues to be a challenge for the treatment of brain tumors. Decreases in apparent diffusion coefficient (ADC) have been shown to correlate with an increase in tumor cellularity<sup>1-5</sup>. Graded functional diffusion maps (gfDM)<sup>3</sup> result from the subtraction and thresholding of ADC maps from multiple time points. In this study we analyze an invasive glioblastoma brain tumor in-vivo, and validate biomarkers for blood volume, and increased cellularity with ex-vivo brain tissue.

**METHODS** One 70 year old male patient with an unoperated brain tumor was evaluated for this study. Patient initially presented with a WHO grade 2 astrocytoma, as determined by stereotactic biopsy. The patient underwent radiation treatment, with concomitant Temodar. After six cycles of chemotherapy, the patient radiographically progressed, and began treatment with Avastin. Following 3 cycles, subtle radiographic progression was noted, and Accutane was added to the treatment regimen. Following further progression, Temodar was reintroduced. Throughout treatment 15 MRI sessions, including rCBV, were gathered over the course of 2 years and 4 months. rCBV was processed as previously published<sup>6</sup>. The patient's final imaging study was acquired 17 days prior to death. Patient consented to donation of his brain to our research institution. The brain was fixed in formalin solution and sliced two weeks later using a custom-made rig designed to allow for slicing in the same axial orientation as the gathered imaging. Histological samples were taken from 4 regions exhibiting hypercellularity on gfDMs and increased rCBV. One control region was also sampled that contained no hypercellularity for comparison (C in Figure 1). Samples were paraffin-fixed and cut into 5um sections. Standard H&E staining was performed.

**RESULTS** Figure 1 shows two slices of MRI-derived rCBV and gfDM data, compared to ex-vivo tissue samples. Regions of histological sampling are highlighted with white boxes and overlaid on the MRI images. Histology of one representative tissue sample is shown on the bottom. We found subependymal tumor spread along the wall of the right posterior ventricle that aligned well with a region of heightened hypercellularity on gfDMs, and slightly increased rCBV. In the control region, no viable tumor was present. In all other samples, neoplastic changes were observed, which included pallasading necrosis, mitotic figures, and dense hypercellularity indicative of a now more aggressive WHO grade 4



glioblastoma.

**DISCUSSION** In this study we validate gfDM metrics of tumor cellularity with ex-vivo tissue. Additional staining and investigation is underway to validate gfDM and rCBV measures, and other biomarkers for brain tumor characterization.

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