In Vivo MR Imaging and Spectroscopy Provides Insight into Malignant Transformation and *IDH*-mutation Status in Diffuse, Low-grade Glioma

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Introduction: Infiltrating low-grade gliomas (LGGs) are a class of central nervous system tumors comprised of malignant neuroglia. Histopathological diagnosis of low-grade (Grade II) or high-grade (Grades III/IV) glioma is performed using criteria set by the World Health Organization (WHO), based on several factors including nuclear atypia, proliferative capacity, cellularity, and tumor neovascularization.[1] While some LGGs will have a more indolent disease course and remain Grade II, many will recur as a higher grade lesion after undergoing malignant transformation (MT). Clinical outcome and treatment sensitivity for LGGs have been linked to the presence of somatic mutations in the Isocitrate Dehydrogenase 1 & 2 (*IDH1/2*) oncogenes, which significantly impact the tumor genome, epigenome, and metabolome.[234]

Objective: Although MR imaging and spectroscopy have played a pivotal role in the treatment and disease monitoring of LGG, the ability to non-invasively assess MT-associated changes in morphology and pathophysiology, as well as *IDH*-genotype has been limited. The goal of this study was to investigate the imaging and spectroscopy profiles of patients with tumors that have histopathological confirmation of MT, as well as those that harbored the *IDH*-mutation, using state-of-the-art MR imaging and spectroscopy (MRSI) techniques.

Methods: 125 patients with an original pathological diagnosis confirmed to be a WHO Grade 2 glioma were included in our IRB-approved study. Patients were recruited immediately prior to resection for suspected recurrence, when progression to higher grade is often observed. They had received prior biopsy or surgical resection and a subset received prior radiation and chemotherapy.

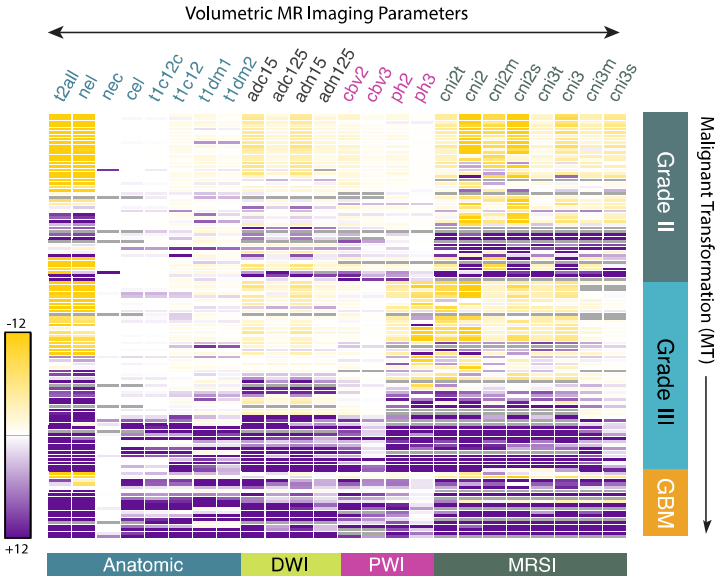
In vivo MR Scans: Preoperative MR studies were conducted at either 1.5 or 3 Tesla. The scans included 6 directional axial Diffusion Weighted Imaging (DWI) with b=1000s/mm2; lactate-edited 3D MRSI with PRESS volume localization; and dynamic susceptibility contrast enhanced Perfusion Weighted Imaging (PWI) with a 3-5ml/s injection of 0.1mmol/kg body weight Gd-DTPA.

Tissue Acquisition: 1-4 issue sample locations were selected in BrainLab navigation software for each patient based on surgically accessible areas that had low Apparent Diffusion Coefficient (ADC), elevated Choline/N-Acetylaspartate index (CNI), or elevated PWI peak height and decreased recovery. Upon surgical excision these tissue samples were immediately sectioned and fixed in 4% formalin, dehydrated by graded ethanols, and embedded in wax using standardized techniques for histopathology analysis and *IDH1* immunohistochemistry using an anti-IDH1R132H antibody. A total of 249 images guided tissues samples were acquired.

Data Analysis: MR parameter values from normal appearing brain tissue (NABT) was defined for each scan using the mode of the histogram from the whole brain minus the region of hyperintensity on T2 weighted FLAIR images. Regions of interest from the lesion were evaluated using in house software and included: the anatomic volumes of T_2 hyperintensity (T2ALL), contrast enhancement (CEL), necrosis (NEC), non-enhancing lesion (NEL), T_1 region > 1.2x NABT in the CEL or T2ALL (t1c12c, t1c12), T_1 region > 1.1x or 1.2x NABT after T_1 difference subtraction (t1dm1, t1dm2); the diffusion volumes of ADC > 1.5x or 1.25x NABT in the T2ALL (adc15, adc125) or in the NEL (adn15, adn125); the perfusion volumes of non-linear Cerebral Blood Volume (CBV) > 2 or 3 (cbv2, cbv3), non-parametric peak height > 2 or 3 (ph2, ph3); spectroscopy volumes of CNI > 2 or 3 in the T2ALL (cni2t, cni3t) or in the whole brain (cni2, cni3), CNI > 2 or 3 overlapping with T2ALL prior to sinc interpolation (cni2m, cni3m) and after upsampling (cni2s, cni3s). Normalized image intensity values were calculated for each lesion for the minimum, 10th percentile, median, 90th percentile, and maximum anatomic, DWI, PWI, and MRSI values. This was repeated at the tissue-target location using 5mm spherical ROIs that were generated for comparison to histopathology parameters. A Wilcoxon rank rum test was used to assess statistical significance of MT and *IDH*-mutation ($p<0.05$). A Pearson correlation test was used to compare tissue level histopathology and imaging parameters.

Results: We report significant intra- and inter-grade heterogeneity of imaging profiles in patients with recurrent LGG. There were significant differences in DWI, PWI, and MRSI imaging volume parameters for lesions that had undergone MT, while DWI/PWI distinguished *IDH*-mutation status, and DWI/MRSI volumes distinguished oligodendroglioma histology (Table 1). Figure 1 demonstrates the global differences of abnormal imaging volume parameters across all tumor grades. The intensities of parameters from the anatomic imaging, DWI, PWI, and MRSI were also significantly different for lesions that had undergone MT and MRSI alone was significantly different for lesions with wild type versus mutated-*IDH* (not shown). As expected, there were significant associations between imaging parameters at the tissue target level and histopathology markers that corresponded to the underlying tumor biology.

Conclusions: Our results suggest that multi-parametric, quantitative *in vivo* MR imaging may play a significant role in non-invasively distinguishing MT and *IDH*-mutation for patients with LGG. Future work will focus on development of a machine-learning algorithm that can provide accurate assessment of tumor grade and genotype, with the ultimate goal of improving the clinical management of patients with this diagnosis. **References:** [1] Grier, et al. (2006) Oncologist 11:681-693 [2] Yan, et al. (2009) NEJM 360:765-73 [3] Turcan, et al. (2011) Nature 483:479-483 [4] Dang, et al. (2009) Nature 462:739-743 *This work was supported by the NIH Brain Tumor SPORE Grant P50CA097257*



Malignant Transformation

	parameter	increase/decrease	p value
Anatomic	t2all	increased	$p < 0.001$*
	cel		$p < 0.001$*
	nel		$p < 0.001$*
	nec		$p < 0.01$*
	t1c12c		$p < 0.001$*
	t1c12		$p < 0.001$*
	t1dm1		$p < 0.001$*
	t1dm2		$p < 0.001$*
DWI	adn125		$p < 0.001$*
	adn15		$p < 0.001$*
	adc125		$p < 0.001$*
	adc15		$p < 0.001$*
PWI	cbv2		$p < 0.001$*
	cbv3		$p < 0.001$*
	ph2		$p < 0.001$*
	ph3		$p < 0.001$*
MRSI	cni2		$p < 0.01$*
	cni2t		$p < 0.01$*
	cni2m		$p < 0.01$*
	cni2s		$p < 0.01$*
	cni3		$p = 0.052$
	cni3t		$p < 0.05$*
	cni3m		$p < 0.05$*
	cni3s		$p < 0.05$*

***IDH*-mutation Status**

	parameter	increase/decrease	p value
Anatomic	t2all	increased	$p = 0.077$
	nel		$p = 0.051$
DWI	adn125		$p = 0.067$
	adn15		$p < 0.05$*
	adc15		$p < 0.05$*
PWI	cbv2		$p < 0.05$*
	ph2		$p < 0.05$*
	ph3		$p = 0.065$
MRSI	cni3t		$p = 0.071$

Oligodendroglioma Histology

	parameter	increase/decrease	p value
Anatomic	t1c12	decreased	$p = 0.068$
	t1c12c		$p = 0.076$
	cel		$p = 0.072$
DWI	adn125		$p = 0.056$
	adc125		$p < 0.05$*
MRSI	cni2		$p < 0.01$*
	cni2s		$p < 0.05$*
	cni3		$p < 0.05$*

Table 1. Statistically significant parameters distinguishing MT and *IDH*-mutation status

Figure 1. Heterogeneity of abnormal imaging volume parameters among patients with recurrent LGG. A linear color gradient heatmap demonstrates differences below (yellow) and above (purple) the median value of each imaging parameter. Each row represents a patient scan that was hierarchically clustered within each grade.