Is quantitative T2 sensitive to tumor cell infiltration?

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Introduction: Quantitative analysis of multi-echo T_2 relaxation data (qT_2) has been used to discern micro-compartmental structures in brain. T2 distribution histograms derived from multi-exponential analysis demonstrates anatomical composition of brain and can be used to quantify myelin content in MS patients [1], identify water compartments in MS and PKU pathological tissues [2], and to identify multiple tissue-types in rat glioblastoma tumors [3]. The infiltrative nature of cancers in general, and malignant gliomas in particular, poses a major clinical challenge. Recently, brain tumor initiating cells (BTIC) have been identified in brain malignancies and have been hypothesized to represent the cell of origin for these tumors [4]. We analyzed 5 mouse brains in vivo inoculated with BTIC to characterize the changes in T2 distributions corresponding to different types of pathological regions of heterogeneous tumors, which were then compared with ex vivo histological stains.

Methods: *Subjects:* BTICs established from fresh surgical specimens were injected into the brains of 5 SCID mice [5]. Tumors were allowed to establish and mice were scanned on day 92 followed by immediate sacrifice. The brains were fixed, paraffin embedded, cut into 5 µm thick slices, and prepared for immunohistochemical analysis. Slices within the MR scan were stained with a neural specific progenitor marker (nestin) that can selectively show tumor cell cytoskeleton so that the density of the stain represents tumor cell population. *MRI Analysis:* Mice were scanned using a single-slice qT_2 acquisition, through the site of BTIC injection, on a 9.4T Bruker console. qT_2 acquisition parameters were as follows: 128 echoes, 5.5ms echo spacing, 3000ms TR, 0.75mm slice thickness, 1.92x1.92cm^2 FOV, matrix 128x128 pixels, 4 averages. After segmenting out the brain, NNLS was performed on a pixel-wise basis [6], creating T_2 distributions for each pixel within the brain. The T_2 times were logarithmically spaced between 2.75 and 1056ms. The decay curves were truncated to 96 echoes from 128 echoes in order to avoid an accumulation artifact. The qT_2 data were visualized and analyzed using in-house software (qT_2-View), which allows brain to be segmented according to T_2 values specified from the T_2 distribution histogram. For each mouse, 4 T_2 bands (Fig 1C) were defined as follows: 18.39-45.24 ms (normal appearing peak, T_2 band 1, dark red), 47.56-52.56 ms (secondary peak, T_2 band 2, red), 55.25-95.77 ms (shoulder of secondary peak, T_2 band 3, orange), and 100.68-524.35 ms (prolonged peak, T_2 band 4, yellow). For each T_2 band, an area fraction map and a gmT2 map were computed. Next, an ROI was drawn on the gmT_2 map including regions highlighted for each T_2 band as shown color-coded in Fig 1A. A local T_2 distribution histogram was created using data only within the ROI. The local histogram was divided up into the 4 T_2 bands described previously and area fractions and gmT_2 times were calculated.

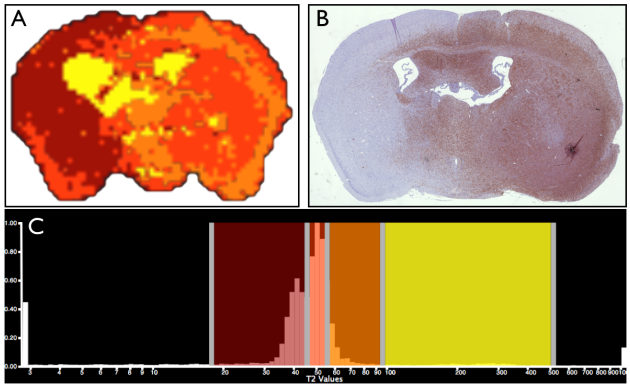

Fig 1: Segmented and color coded gmT2 map (A), nestin stained histology (B), and color-coded T2 distribution histogram (C).

	Area Fraction				Geometric Mean T_2			
	T_2 Band 1	T_2 Band 2	T_2 Band 3	T_2 Band 4*	T_2 Band 1	T_2 Band 2	T_2 Band 3	T_2 Band 4*
ROI 1	66.8 ± 2.4	14.7 ± 6.0	0.5 ± 0.1	3.0 ± 0.6	38.9 ± 0.3	48.3 ± 0.5	66.1 ± 2.6	272.7 ± 12.0
ROI 2	3.8 ± 0.7	48.7 ± 5.0	11.2 ± 2.1	1.9 ± 0.8	31.9 ± 1.5	50.1 ± 0.6	59.3 ± 0.6	237.4 ± 16.2
ROI 3	2.2 ±0.8	16.4 ± 3.0	44.0 ± 3.5	1.2 ± 0.3	30.0 ± 1.0	50.8 ± 0.7	60.4 ± 0.8	228.7 ± 18.2
ROI 4	10.6 ±3.2	30.4 ± 10.4	8.6 ± 3.6	28.4 ± 11.9	38.5 ± 2.5	48.8 ± 0.4	70.3 ± 1.9	293.4 ± 7.0

Table 1: Area fractions and gmT_2 for each T_2 band in 4 ROIs. Standard error shown. *Only present in 3 mice.

Results: The prolonged peak was present in 3 mice. A color-coded T_2 distribution histogram for a mouse is shown in Fig 1C and the corresponding geometric mean T_2 (gmT_2) map is shown in Fig 1A. A nestin stained histology slice is shown in Fig 1B. Table 1 shows the area fractions and gmT_2 times for the 4 T_2 bands defined for each ROI.

Discussion: This study suggests that gmT_2 values lengthen with the presence of tumor cells. The existence of area fractions among different T_2 bands for each ROI demonstrate the multiexponential behaviour due to the heterogeneous nature of the cancer, although the T_2 band defining the ROI predominated the T_2 distribution. The prolonged peak (T_2 band 4), when it was present, had a large intensity peak corresponding to the T_2 band 2. It was only present in 3 mice, which may indicate a specific behavioral pattern of the cancer. Based on the qualitative comparison between segmented gmT_2 maps and histology slides the 4 color-coded regions shown in Fig 1A generated from 4 T_2 bands appear to correspond with varying tumor cell densities in Fig 1B. Through our analysis, we have identified distinct regions with different water compartments within BTIC generated brain tumors. The physiological significance of these distinct regions is not yet known.

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References: [1] Laule *et al.* NeuroImage 40: 1575-80, 2008 [2] Laule *et al*. JMRI 26: 1117-21, 2007 [3] Dortch *et al*. NMR Biomed 22: 609-618, 2009 [4] Singh *et al.* Cancer Res 63:5821-5828, 2003 [5] Kelly *et al*. Stem Cells 27:1722-1733 [6] Bjarnason *et al* MRM 2009 (in press)