Direct imaging of tumor cellularity using restriction spectrum imaging in a xenograft mouse GBM model

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Purpose

The purpose of this study is the demonstrate how the restricted water signal, as measured with a constant b-value, multi diffusion time diffusion experiment, provides a novel contrast mechanism for identifying cancer cells *in-vivo*.

Introduction

The diffusion weighted imaging (DWI) technique enables measurements and quantification of water mobility probing microstructural properties of biological tissue, and has become a useful tool for assessing information about the underlying pathology of cancerous tissue. We have previously demonstrated how a multi-b-value diffusion experiment at a fixed (long) diffusion time on a clinical scanner can be used to separate hindered and restricted water pools in tumors and that the restricted water fraction provides superior tumor contrast-to-noise compared with traditional ADC [1]. In a follow-up Monte Carlo study [2], it was determined that the high conspicuity for aggressive cancers likely stems from high nuclear volume fractions of individual cells and correspondingly low AR_2 for intracellular water. In this study, we test these findings directly in a mouse model of GBM, using a modified *in-vivo* imaging protocol that manipulates both b-values, echo time (TE), and diffusion time (Δ).

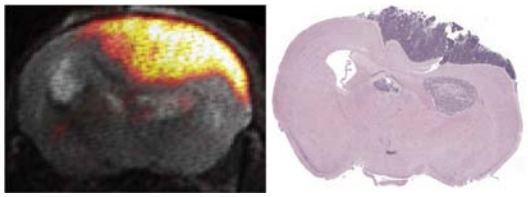

Figure 1: Left; Restricted signal measured as the signal difference between Δ=11ms and Δ=60ms, Right; Corresponding H&E stained histology slide.

Methods

A mouse was injected with a patient derived GBM cell line and imaged *in-vivo* using T_2 weighted and restriction spectrum imaging (RSI). The RSIs were acquired using single shot EPI, 8 b-values of 500-4000s/mm2 at a 500 s/mm2 interval, TEs of 59ms, 79ms, 100ms and 120ms at a fixed Δ = 40ms, and Δ of 11s, 20ms 40ms and 60ms at a fixed TE = 79ms. After imaging, the mouse was sacrificed, its brain was fixed, embedded, sectioned and stained with hematoxylin and eosin (H&E) stain. All diffusion images were coregistered to the T2 weighted space and rotated according to the histology slides.

Results

Figure 1 shows how the signal difference between Δ=11ms and Δ =60ms may act as a direct measure of restricted diffusion and a cancer marker. Figure 2 shows the b-value dependence (x-axis) and the TE (upper) and the Δ (lower) dependence (y-axis) on the diffusion signal. Figure 3 demonstrate the increasing signal contrast between two regions of interest, tumor (blue) and normal appearing brain matter (nabm, red) as a function of TE (a) and Δ (b) at b=4000s/mm^2.

Main findings:

1. The restricted signal depends on the average AR_2 of the cell
2. By changing Δ, the restricted diffusion can be measured directly.

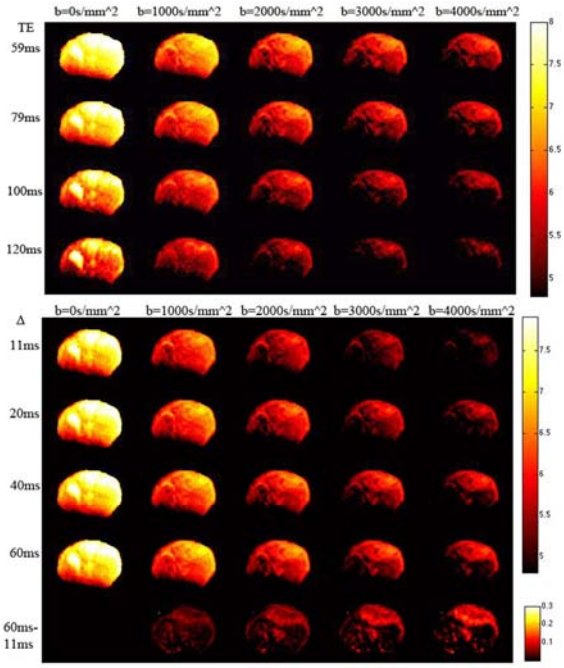

Figure 2: TE dependence (upper, y-axis), Δ dependence (lower, y-axis) and the b-value dependence (x-axis) on the ln of the signal intensity. Bottom row show the difference between Δ=11ms and Δ=60ms for each b-value.

Discussion

In this study we show that changing the Δ while keeping the TE constant, allows for isolation of the restricted signal, and that the restricted contrast is b-value dependent (bottom row of Figure 2). With increasing b-values the signal from the fast extracellular water compartment is increasingly attenuated. At high b, only signal from the restricted pool is left. At long Δ, all intracellular water will experience the restriction boundaries, while at short Δ they do not. By subtracting the signal intensity of short from long diffusion time, the restricted diffusion can be measured directly. Further we show that manipulating TE at a fixed (long) Δ can be used to improve the conspicuity and specificity of the tumor signal. This proves the AR_2 dependence on the RSI signal postulated by the Monte Carlo simulation [2]. The elevated signal from the tumor compared to the normal tissue at high b and long Δ reflects the greater emphasis on the restricted water fraction for cells with larger nuclei (as a percentage of the total cell volume) due to the reduced effective R_2 for intracellular water.

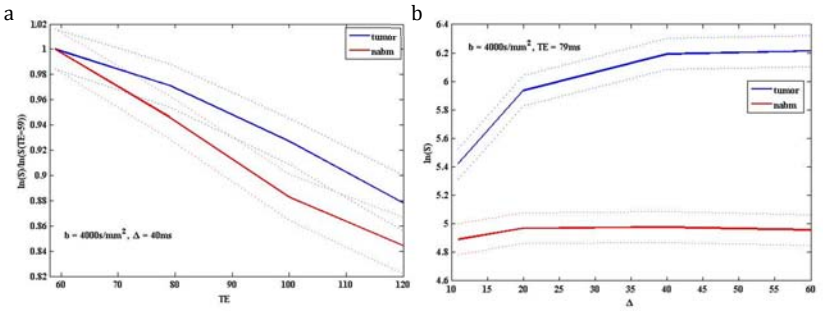

Figure 3 The natural log of signal intensity (S) in the tumor (blue) and the nabm (red) as a function of TE (a) and Δ (b) at b=4000s/mm^2, ln(S(TE)) is normalized to ln(S(TE=59)) to better visualize the tissue contrast.

References: 1. White, N.S., et al., *AJN*. 2012 2. White, N.S., Dale AM. *MRM* (In Press)