Early detection of treatment-induced apoptosis in tumors using temporal diffusion spectroscopy MRI

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Target audience: Researchers interested in MR cancer imaging and diffusion MRI.

Purpose: The restoration of apoptosis in cancer cells is a critical strategy in the development of novel anti-cancer therapies, and the detection of apoptosis may provide an early way to assess tumor response to treatment. However, the in vivo detection of apoptosis is challenging, and none of the current available imaging methods have proven robustly successful in practical applications. Diffusion MRI has been suggested as a possible non-invasive means to monitor apoptosis[1]. However, conventional diffusion MRI uses relatively long diffusion times, making it sensitive to cellularity but relatively insensitive to changes at intracellular scales. Apoptosis is a highly regulated, programmed cell death in which plasma membrane integrity is maintained, at least in early stages. Therefore, conventional diffusion MRI is limited to detecting only late stages of a tumor response to an effective treatment, after there are frank changes in cell density. However, apoptosis at early stages is associated with significant intracellular microstructural variations, such as nuclear fragmentation and cytosolic condensation, and with overall cellular shrinkage, so detection of these specific changes may lead to an early and more accurate detection of apoptosis. Here we show that temporal diffusion spectroscopic MRI, a technique that is capable of characterizing microstructural variations in tumors across intracellular to cellular length scales[2], can provide an early, non-invasive and specific detection of the microstructural variations associated with treatment-induced apoptosis.

Methods: *Theory*: Temporal diffusion spectra are acquired using oscillating diffusion gradients to replace conventional bipolar gradients, and short effective diffusion times can be readily achieved with only moderate frequencies. More importantly, by tuning oscillating frequencies, temporal diffusion spectra can be obtained to reveal microstructural information from short (intracellular) to long (subcellular) length scales.

In vivo experiments: Two types of human colon cancer cells[3], DiFi (responder) and HCT116 (non-responder), were used to create tumors in mice. Animals were treated i.p. with either cetuximab (n=10) or PBS (drug vehicle) (n=8) for 8 days (10 mg/kg, every other day). Diffusion-weighted images (DWI) were collected before, and after 4-day and 8-day treatments. The apparent diffusion spectra over frequencies (f) from 50-350 Hz were obtained with two b-values (0 and 400 sec/mm^2), while the ADC at 0 Hz was estimated by a conventional pulsed gradient sequence with diffusion time = 48 ms. Immunohistochemistry (Annexin V) staining confirmed significant apoptosis occurred at Day 4.

Data analysis: Tumors were modeled as tightly-packed spherical cells, and the entire ADC spectra of each tumor were fit to the equation: $ADC(f) = ADC_{res}(d, D_{in}\text{-}D_0) + D_0$, where ADC_{res} arises from intracellular space with apparent cell size d and intracellular diffusion coefficient D_{in}, while D_0 is associated with the extracellular tortuosity.

Results: Figure 1 compares the percentage changes of tumor volume and three fitted spectral parameters: D_0, D_{in}, and d. For the cetuximab-treated DiFi mice, the average tumor size was decreased at Day 8 ($p<0.05$), indicating the treatments were effective. HCT116 tumors continued to grow markedly, as expected. The apparent cell size d of DiFi tumors decreases significantly at Day 4 ($p=0.01$) and 8 ($p=0.002$), corresponding to treatment-induced cellular shrinkage. A significant decrease of D_{in} ($p=0.001$) at Day 4 indicates slower diffusion inside the treated tumor cells, probably corresponding to the cytosolic condensation and nuclear fragmentation. D_0 for both treated DiFi and HCT116 tumors showed significant changes ($p<0.05$) at both Day 4 and 8, indicating that D_0 does not have sufficient specificity for the detection of treatment response. More importantly, conventional diffusion MR sequences did not report significant changes at Day 4, indicating that the early stage of apoptosis was not captured.

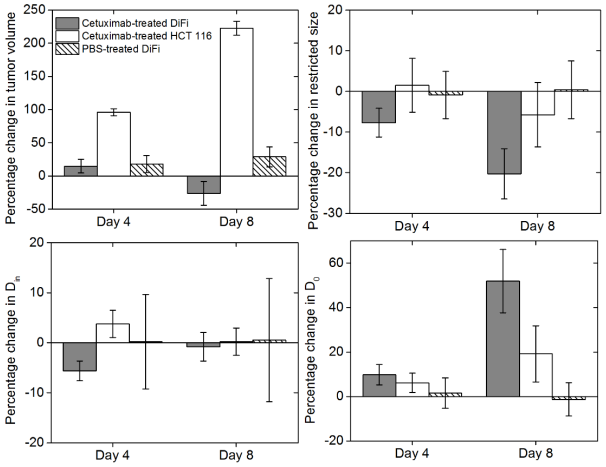

Figure 1. Percentage changes in tumor volume (upper left), and three fitted parameters: D_0, D_{in}, and d (apparent cell size).

Conclusion: These findings suggest that key microstructural features of apoptosis at early stage, such as cell shrinkage, nuclear fragmentation and cytosolic condensation, can be detected by temporal diffusion spectroscopy, while conventional diffusion MRI detects only later stages of apoptosis when cellularity changes. Therefore, temporal diffusion spectroscopy is potentially an earlier and more specific indicator of treatment-induced apoptosis.

References: 1. Brauer M. Prog Neuropsychopharmacol Biol Psychiatry 2003;27:323–31.

2. Xu J, et al. PLoS One 2012;7:e41714.

3. Manning HC, et al. Clin Cancer Res 2008;14:7413–7422.