In vivo MR diffusion tensor study of bacteria infiltration in murine tumor

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Introduction Fast growing solid tumors usually contain hypoxic thus less vascularized regions that limit the efficacy of both chemo- and radiation therapy due to poor drug delivery and lack of O_2 to sensitize the cytotoxic effects of ionizing radiation. Bacterial-based approach has shown to eliminate animal tumors when combined with chemotherapeutic drugs; however, mechanisms of bacterial accumulation in tumors are not known (1). Previously we reported (2) MR apparent diffusion coefficient (ADC) study of Lewis lung carcinoma (LLC) infiltrated with genetically-engineered bacteria in C57B/6J mice. Here we present an MR diffusion tensor study of the LLC tumor treated with a different bacteria strain.

Method Lewis lung carcinoma LLC cells (ATCC) were inoculated subcutaneously on the flank of mouse C57B/6J. Two weeks after tumor implantation, genetically altered *Salmonella typhimurium* were injected into the tumor. Six days after bacteria injection, 6 diffusion weighted and 1 spin echo images were acquired on a Bruker 7T/31cm Biospec imager (ParaVision 3.0.2) with the Bruker sequence DWI_SE. Imaging and diffusion acquisition parameters were TE/TR = 37.3ms / 2000 ms, phase encoding steps = 128, slice thickness = 1 mm, FOV = 2.5 cm, G= 150 mT/m, Δ = 18 ms, δ = 6 ms, and diffusion-weighting b = 927 s/mm^2, with negligible imaging and cross-term b values, the latter is due to a slightly angled slice orientation. The 6 diffusion weighting gradients were applied (3) along (x y z) directions of (1 0 1), (-1 0 1), (0 1 1), (0 1 -1), (1 1 0), and (-1 1 0). Histograms, Gaussian curve fitting, Mean diffusivity (MD) and Fractional anisotropy (FA) maps were calculated in Matlab 7.0.1 (Math Works, Inc.) on a PC Windows platform.

Results and Discussion Bacteria were found accumulated near the center of the tumor, modifying the tissue dramatically (Fig 1a), manifested as dark regions where protons move about more freely in a less restricted environment. The MD map (Fig 1b) shows that the two invaded areas (bright) have higher diffusion coefficients (~1 – 1.4 x 10^{-3} mm^2/sec), whereas the rest of the intact tumor appears relatively uniform (~0.5 x 10^{-3} mm^2/sec). This diffusion coefficient agrees well with an earlier result of apparent diffusion coefficient (ADC). In the intact tumor tissue FA is ~0.25 - 0.65 (Fig 1c). In the lower right bacteria-invaded region with a greater MD (brighter), the corresponding FA's are lower (~0.15, darker), indicating more isotropic yet faster diffusion due to LLC tissue destruction. Interestingly, the boundary surrounding the two regions shows the lowest MD (< 0.1 x 10^{-3} mm^2/sec) but the highest FA (> 0.9), indicating a slowest yet the most anisotropic diffusion (the negative MD's are likely due to truncation or fitting error in computation). This is the interface between the invaded and the intact tumor areas where immune response (macrophages), bacteria, and tumor cells interact. To quantitatively study diffusion map in various areas we present ADC results of a follow up study of the genetically altered *Salmonella typhimurium* invading LLC tumor on mouse CB17 SCID (2). Fig. 2 and Table 1 show ADC histograms and their Gaussian fits in four zones - intact, heavily-, lightly- modified tumor tissues, and interface area between modified and intact tissues. The mean ADC (~1.74 x 10^{-3} mm^2/sec) in the heavily-modified zone is three times greater than that (~0.58 x 10^{-3} mm^2/sec) in the intact tissue. Further diffusion studies on this bacteria-tumor system, combined with simultaneous biological investigations, may reveal important physiological mechanism of tumor tissue modification in bacteria-based therapy.

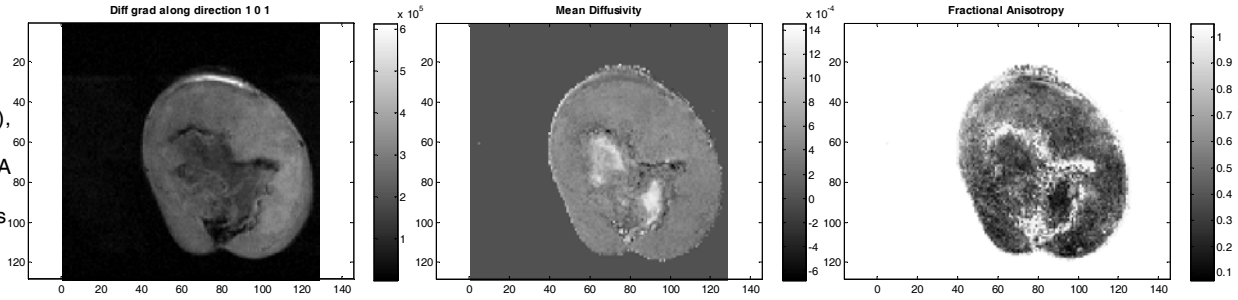
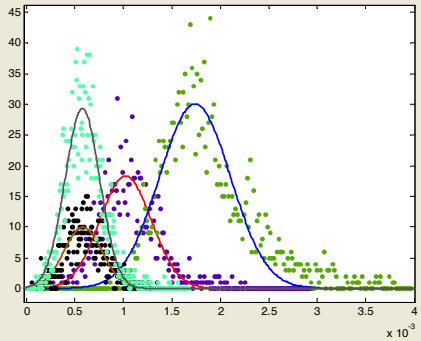


Fig. 1a Diffusion weighted image with gradients applied along readout and slice directions (1 0 1), arbitrary units. Figs. 1b MD map and 1c FA map. Fig. 2 ADC histograms of 4 zones of an LLC tumor on mouse CB17 SCID after theraputic *Salmonella* invasion.

Fig 1a **Fig 1b** **Fig1c**

Table 1 Results of Gaussian fits of 4 ADC histograms in Fig. 2 **Fig 2**

Zones in Figure 2	Description	ADC mean (x 10^{-3} mm^2/sec)	ADC std dev (x 10^{-3} mm^2/sec)
1 (red curve)	lightly modified tumor tissue	1.02	0.176
2 (blue)	heavily modified tumor tissue	1.74	0.290
3 (orange)	interface area	0.582	0.138
4 (gray)	intact tumor tissue	0.576	0.127



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