In vivo MR Diffusion Investigation of Murine Tumors with Bacteria Infiltration

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Introduction. Hypoxic tumors contain poorly vascularized regions that limit the efficacy of chemo- and radiation therapy due to poor drug delivery and lack of O_2 to sensitize the cytotoxic effects of ionizing radiation. Anaerobic bacterial-based approach has shown to eliminate animal tumors when combined with chemotherapeutic drugs (1). In most cases, the mechanisms of bacterial accumulation in tumors are unknown. We have observed that the tumor lactate level drop initially after administration of the attenuated *Salmonella typhimurium*, and reached up to the original level within 24 hours (2-3) . In this presentation, we report preliminary results from an *in vivo* MRI diffusion investigation of Lewis lung carcinoma LL/2 infiltrated with genetically-engineered bacteria *E. Coli* strains in C57B/6J mice.

Methods. Lewis lung carcinoma LL/2 cells (ATCC) were inoculated subcutaneously at one foot of the C57B/6J mice. Two weeks after tumor implantation when the tumors grew to ~9-11 mm diameter, genetically altered *E. Coli* DH5α or JM109 were injected into the tumors. 48 hours after *E. Coli* injection, five diffusion weighted images were acquired on a Brucker 7T/31cm Biospec imager (ParaVision 3.0.2) with the Brucker diffusion-weighted spin echo sequence, DWI_SE. The intubated mice were under fully oxygenated environment of 40% N_2 and 60% O_2. A gap resonator (21 mm diameter) was constructed in-house to fit the tumor mass with optimal filling-factors. TR/TE = 2000ms / 30 ms, slice thickness = 1mm, and FOV = 2 cm. The diffusion acquisition parameters were Δ=11 ms, δ=6 ms, b factors 4, 88, 341, 1468, 2874 s/mm^2. Apparent diffusion coefficient (ADC) was calculated in Matlab (Math Works, Inc.) on a Silicon Graphics 3200 System.

Results and Discussions. The diffusion-weighted images and the ADC maps were shown for two LL/2 tumors injected with *E. Coli* JM109 stain made to carry Hb and DH5αstain to carry Met protein (Fig. 1-2). The Gram-staining has shown that the *E. Coli* DH5αbacteria infiltrated into the tumor tissue, but the *E. Coli* JM109 (Hb) bacteria did not. The T_2-weighted image (Fig. 2a) (at zero gradient strength) presented anatomical heterogeneity of the tumor tissue that eliminated the *E. Coli* JM109 stain; on the other hand, the DH5α T_2-weighted image (Fig. 1a) appeared to be more homogeneous with dark spots in the center region. The five larger dark spots in all images are bones of mouse toes. In histology slices, the DH5α were found in the low cellular area in the tumor center, and they did not reside at the outer rim with high density of tumor cells. This seems reflected by the MRI images—a MR heterogeneous C57B/6J-LL/2 tumor did not carry the *E. Coli* JM109 strain, whereas the tumor with infiltrating *E. Coli* DH5α showed uniform tissue characteristics. The diffusion-weighted images from 1b to 1e demonstrate progressively low ADC areas (brighter) vs. the high ADC areas (darker) as the diffusion-weighting increases. The diffusion map Fig.1f shows low ADC regions in the interior of the tumor, not observable in the anatomical image Fig. 1a. The ADC maps (1f and 2f) show distinctive features not apparent in their anatomical counterparts, with higher ADC in less uniform areas and lower ADC in the more uniform regions. Similar ADC histograms were obtained to be Gaussian for both mice, with mean ~ 0.56 x 10^{-3} mm^2/s.

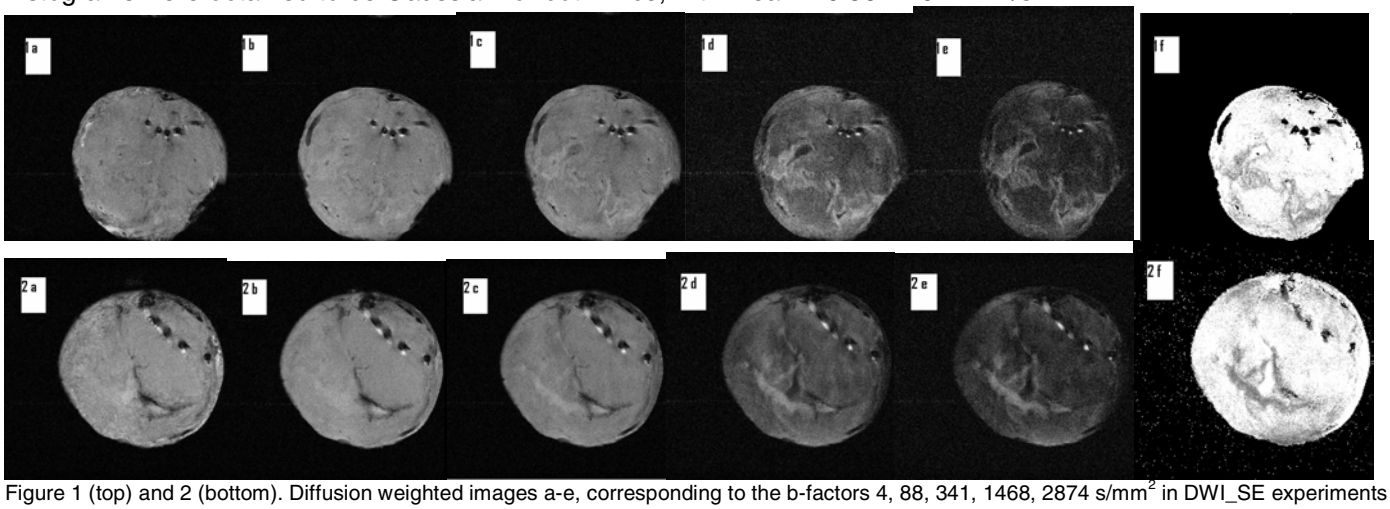

Figure 1 (top) and 2 (bottom). Diffusion weighted images a-e, corresponding to the b-factors 4, 88, 341, 1468, 2874 s/mm^2 in DWI_SE experiments of the DH5$\alpha$and JM109 injected LL/2 tumors in C57B/6J mice, respectively. The diffusion maps are displayed in f.

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