Bacterial-Based Magnetic Resonance Contrast Agents

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INTRODUCTION. Cancer treatment using bacteria cured ~10% patients in the clinical trial about half century ago. The issues of bacterial infection, which stopped the clinical practice, have been recently addressed by knocking out the genes responsible to produce the bacterial toxins using *Salmonella typhimurium* as an example. The attenuated *Salmonella typhimurium* posses high tropism in tumor tissues in animal models including primates.[1] Tumor-specific accumulation was also observed with several other bacteria and viruses.[2] The attempts to selectively deliver anti-cancer drugs to human cancerous tissues using microorganisms have motivated our previous investigation on the mechanisms of bacterial accumulation in tumor tissues. We found that the attenuated *Salmonella typhimurium* caused dramatic tissue lactate drop during the initial phase of bacterial growth in the Colon 38 murine tumor model.[3, 4] Compared with the wild-type *Salmonella typhimurium*, the attenuated *Salmonella typhimurium* strains reduced virulence more than 10,000-fold, thus possessing an excellent safety profile.[5] In a phase I clinical trial, however, only a small number of human melanoma metastasis accumulated the attenuated *Salmonella typhimurium* strain VNP20009, indicating perhaps a less immune tolerance to the bacteria in human than in animals. Currently, we are developing non-invasive MR molecular imaging methods to study bacterial colonization in tumor microenvironment. Here we report a bacterial-based MR contrast agent constructed using Gd(DTPA)-conjugated anti-Salmonella antibody. The live bacterial-based MR contrast agents may be used to detect cancer or to produce proteins that can activate the death pathways of cancer cells.

METHODS. *Salmonella-Gd contrast agent*. To construct the Gd-conjugated anti-Salmonella mAb**,** 20mg of Avidin (Inovatech, Abbotsford, B.C. Canada) in 1ml of Sodium Bicarbonate buffer was allowed to react at pH 8.4 for 24 hours with 10.7 mg of DTPA (Sigma, St. Louis MO). The Avidin-DTPA conjugate solution was filtered using Amicon Ultra-15 10K centrifugal Device (sigma-aldrich) at 3100 rpm for 20 minutes to remove excess DTPA.[6] Gadolinium(III) chloride (Sigma, St. Louis MO) (11.2mg) was then added to allow 24 hours for Gd-chelating reaction at 4°C. The Avidin-Gd conjugate was then filtered again at 3100 rpm for 20 minutes. The attenuated Salmonella typhimurium (1ml) from ATCC (VNP20009) in 5 ml LB/Amp media was mixed with 0.05 ml of Biotinylated anti-Salmonella antibody (OEM concepts, Toms River, NJ) before allowing 0.2 ml of Avidin-Gd conjugate to react with the bacteria. The final Salmonella-Gd solution was centrifuged for 2 minutes at 300 rpm to remove the supernatant containing any free mAb and Gd-Avidin. The Salmonella-Gd pellet was washed with media 5 times. The supernatant was collected separately from each wash for MRI experiments.

MR Phantoms. We constructed a three-compartment phantom for MRI experiments (Fig. 1). A 20 cm long dialysis membrane tubing with a cutoff molecular weight of 6-8,000 (Spectrum Laboratories, Rancho Dominguez, CA) was put in 10 ml distilled water for 30 minutes to remove sodium azide. The Gd-conjugated Salmonella in media was placed in the sealed dialysis tubing, which was then placed in a loosely capped 50ml polypropylene conical tube containing 35ml LB/Amp media. This conical tube was then centered in a 500ml beaker filled with distilled water for MRI experiments on a GE 3T MRI scanner. The Gd-conjugated Salmonella was only in the inner membrane compartment. Water and other small molecules in the cell media could exchange freely between the two inner compartments. For MRI experiments on a Bruker Avance 500 spectrometer, the plastic tube, which collected the supernatant after centrifuge spinning of the Gd-conjugated Salmonella into a pellet, was placed into a saline solution to compare MRI contrasts of the supernatant and saline.

MRI instruments. The MRI experiments were carried out on both GE 3T scanner and Bruker Avance 500 vertical bore small animal imaging system. The interleaved multi-slice images (256 x 256) were obtained on the GE 3T scanner using gradient echo (GRE) or spin echo (SE) imaging sequences with standard GE parameters: TE = 4ms, TR = 250 ms for GRE experiments; and TE = 100 ms and TR = 1000 ms for SE scanning. Slice thickness = 3 mm, FOV = 20 cm unless specified otherwise and NEX =2. On the Bruker 500 system, TE = 3.5 ms and TR = 200 ms were used in the GRE sequence.

RESULTS AND DISCUSSIONS. T_1- or T_2-weighted MRI images were acquired from GRE and SE sequences, respectively, on the GE 3T scanner from the three-compartment phantom (Fig. 1). Enhanced MR contrast was observed in the inner compartment, where the Gd-conjugated Salmonella were confined in the dialysis membrane tubing sealed at the bottom with rubber band (causing the susceptibility darkening in the images). To confirm that the MRI contrast enhancement was not caused by the free Gd-conjugated anti-Salmonella antibodies (without binding to the bacteria), we centrifuge span the Salmonella-Gd into a pellet and collected the supernatant in a test tube, washed the Salmonella pellet with LB/Amp media, and repeated the centrifuge-wash procedure 5 times. The supernatant from the 4th repeat was imaged against a saline solution on the Bruker Avance 500 MR spectrometer. No MR contrast-enhancement was observed in the supernatant compartment, indicating that the MR contrast enhancement observed in the inner bacterial compartment of the GE 3T phantom was originated from the Gd-conjugated bacteria. FACS analysis is in progress to count the average number of the bacterial surface receptors for the anti-Salmonella mAb. *In vivo* MRI experiments also will be performed using animal tumor models to detect primary and metastatic lesions with the Salmonella-based MR contrast agents.

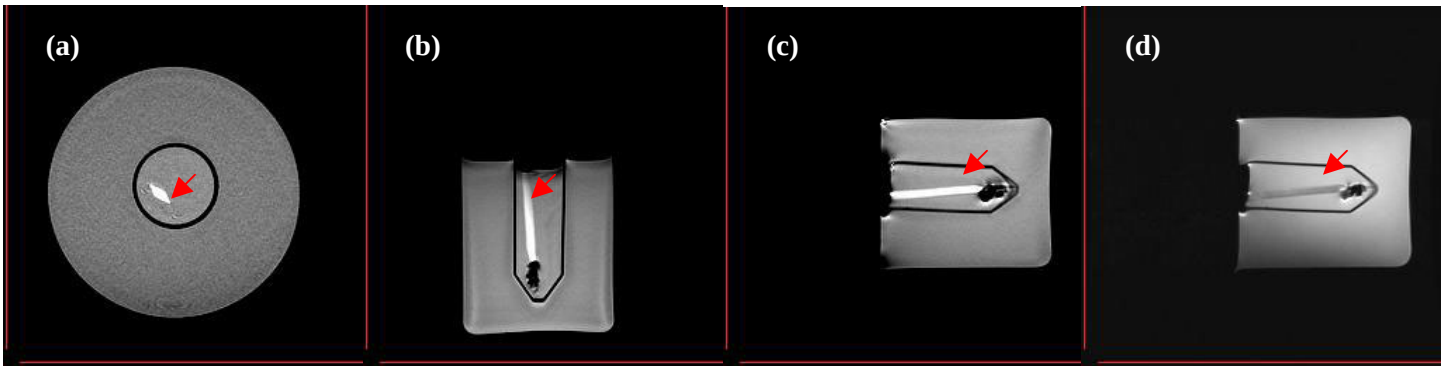

Fig. 1 The attenuated Salmonella typhimurium VNP20009 tagged with Gd(DTPA) by anti-Salmonella monoclonal antibody. Enhanced imaging contrast was observed in the inner membrane compartment containing the bacteria. The T_1-weighted images from GRE with positive contrast were acquired on a GE 3T scanner in: (a) coronal (FOV = 12 cm) (b) axial and (c) sagittal planes. (d) A T_2-weighted spin echo image with a negative contrast of the inner Salmonella-Gd compartment.

CONCLUSIONS. The attenuated *Salmonella typhimurium* were conjugated to Gd(DTPA) using the anti-Salmonella antibody. The preliminary phantom studies have demonstrated the feasibility of visualizing VNP20009 strain in tumor tissues. The live Salmonella-based contrast agents will be used to study the selective bacterial accumulation in tumor tissues. Cancer diagnosis using the bacteria carrying the MR contrast agents also will be explored in animals and in clinics.

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