In Vivo Detection of Early Colorectal Tumors in Mice Using Contrast-Enhanced Magnetic Resonance Imaging

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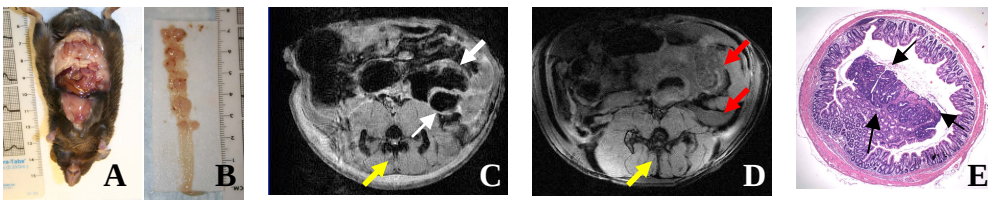
Abstract: We have developed a novel class of contrast agents that contain VO^{2+}-chelated organic ligands (VCs) for magnetic resonance imaging (MRI). These contrast agents provided excellent T_1 and T_2^* contrasts compared to that of Gd-DTPA in high-resolution MR images of rodent tumors. We have demonstrated that these contrast agents are taken up by highly glycolytically active colon cancer cells, and activate kinases involved in glycolysis. These results provide the basis for *in vivo* MRI studies for early detection of colorectal tumors in mice. Here we provided direct *in vivo* MRI experimental evidence with control and tumor-bearing mice that small colorectal tumors of ~1 mm in size can be detected; and MR images can be correlated with immuno-histological images. We have also carried out MRI study after injecting contrast agents, and found that the uptake of contrast agents in tumors increased significantly compared to that of muscle.

Introduction: Inflammatory bowel disease including ulcerative colitis is characterized by persistent or recurrent inflammation that can lead to colon cancer. The most effective therapeutic outcome can be achieved at an early stage of disease. Early detection and more accurate identification of malignancy could improve patient prognosis and help target appropriate therapies. Animal models have been widely used to study the pathogenesis and potential therapies of colitis and colon cancer associated with colitis. However, imaging methods that can evaluate colitis and early colorectal cancers in murine models are not available. Here, we developed improved MRI methods for detecting colitis and monitoring the progression from colitis to cancer in a clinically relevant model in mice.

Methods: To demonstrate the uptake of VCs in cancer cells, we have carried out *in vitro* MRI and atomic absorption studies with intracellular extracts using HCA-7 and Caco-2 cells derived from human colonic adenocarcinomas. Each VCs was added to a final concentration of 100µM, 250 µM, or 500 µM and cells incubated for 5 min. The cytosolic fraction of cell extracts was collected for MRI and atomic absorption studies. We examined AKT (protein kinase B) and ERK (extracellular signal receptor kinase) activations by Western blotting using antibodies to phospho-active AKT and ERK. For *in vivo* MR colonography using a 9.4 Tesla Bruker scanner, we used a mouse model with carcinogen-induced colorectal tumors. Our model recapitulates many of the clinical, histological, and molecular features of human colonic inflammation and colon cancers. Male C57Bl6/J mice were given intraperitoneal azoxymethane (AOM) with a dose of 10 mg/kg of body weight. One week later they received 2.5% dextran sulfate sodium (DSS) in the drinking water for 5 days. Mice bearing colorectal tumors were imaged 15 weeks after AOM/DSS treatment. High-resolution MR images were acquired by gradient echo pulse sequences using multiple slices with 1 mm slice thickness in the coronal and axial orientations.

Results: These results from *in vitro* MRI and atomic absorption studies demonstrate there is intracellular uptake of VCs into colon cancer cells. Analyses of Western blots of phospho-active AKT (p-AKT) and p-ERK in HCA-7 colon cancer cells treated with VCs showed the expression levels of p-AKT and p-ERK increased in a dose dependent manner. The figure below illustrates the following. In panel A, we illustrate a male C57Bl6/J mouse of about 33 g. An abdominal incision allowed inspection of the opened peritoneal cavity. The excised colon (after cleaning) with tumors is shown in panel B. In panels C and D, we illustrate MR images that were taken in axial planes for a control mouse and a tumor-bearing mouse, respectively. The MR images were matched according to spine (yellow arrows). In panel C, white arrows indicate the air filled colon; and in panel D, the red arrows indicate space occupying colorectal tumors of ~1 mm in size. In panel E, we illustrate an immuno-histological slice of the same colorectal tumor, indicated by three black arrows.



(**A**) Open abdominal cavity of a mouse. (**B**) Colorectal tumors. Axial views of MR images of a (**C**) control mouse and (**D**) a tumor-bearing mouse. (E) Distal colon with tumors after H&E staining.

Discussion: Results from cell signaling and *in vitro* MRI and atomic absorption studies demonstrate that VCs accumulate intracellularly in cancer cells, which provide the basis for *in vivo* DCEMRI studies for early detection of colorectal tumors in mice. Correlations of MR images with histological images are also documented. Further DCEMRI studies are in progress. [Supported by grants from the American Cancer Society, Illinois Division, and National Institutes of Health].