Detection of Colonic Tumorigenesis *In Vivo* and Monitoring of Local Colonic Tumor Invasion and Metastasis in Mice using Magnetic Resonance Imaging

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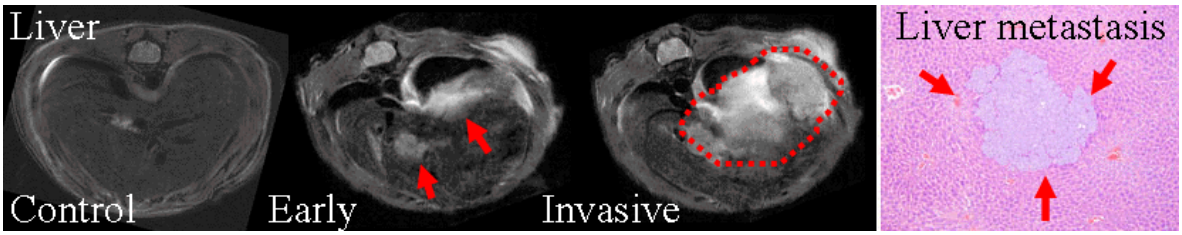
Abstract: Improved methods for the early detection and accurate characterization of cancer are highly desirable, since they could allow cancer treatments to begin at an earlier, more easily managed stage. Dr. Bissonnette and his co-workers at the University of Chicago have developed a novel mouse model of colon cancer metastasis using cecal implants of colon cancer cells. Our primary objectives were: (1) non-invasive detection of cancer and (2) characterization of early colonic cancer and its invasion and metastasis into liver and lungs. Dynamic contrast enhanced MRI (DCEMRI) datasets using Gd-DTPA and high-resolution conventional *in vivo* MR images of cecum, liver and lungs in mice bearing tumors were acquired to obtain functional and anatomical information of early malignant invasive lesions and tumors.

Introduction: Epidermal growth factor receptors (EGFR) and ligands controlling colonocyte growth are frequently over-expressed in colon cancers. We have shown for the first time by both pharmacological (Gefitinib) and genetic approaches (the inhibitory "Wa2" EGFR mutation) that EGFR plays an important role in sporadic and inflammatory colonic tumorigenesis. We now have elegant models to directly address stromal cell/epithelial cell cross talk in both tumorigenesis and metastasis. The primary goal of this project is to develop an *in vivo* MRI method for detecting early colonic cancer invasion and distant metastasis in a murine model. To the best of our knowledge, this is the first report of *in vivo* detection by MRI of the non-invasive detection of unlabeled colon cancer cells implanted orthotopically into the cecum to monitor local invasion and distant metastasis to liver and lungs.

Methods: Dr. Bissonnette *et al*. have adopted a surgical orthotopic implantation model to place highly metastatic HCT116 human colon cancer cells into the cecum of an immunotolerant *Rag1* mouse. These cells invade locally within 1-2 weeks and metastasize in the liver (and lungs) within 4-6 weeks. Using a 9.4 Tesla Bruker MRI scanner, *in vivo* MR images were acquired with respiratory gating for higher spatial resolution. Spin echo and gradient echo images with an in-plane resolution of 100 microns were acquired both along the coronal plane and along the axial plane to cover the range from lungs to cecum. DCEMRI datasets with temporal resolution of 5 sec were acquired after injecting a dose of 0.13 mmol/kg of Gd-DTPA. H&E stains of post mortem tissue slices were carried out to correlate MR images with histological sections.

Results: High-resolution T_1/T_2-weighted MR images showed detailed anatomical views, which were corroborated with high-resolution photographs of mouse organs after incision (following the *in vivo* MRI studies). The figure below shows



representative T_2-weighted MR images of livers for a control, an early, and a highly invasive tumor-bearing mouse. These images were acquired in an axial plane. Early and invasive tumors in the liver are labeled in the figure and indicated by red arrows or by a dotted area, respectively. An H&E section of liver micro-metastasis is also shown in the figure and labeled. The MR image of tumor showing here and labeled as "invasive" is much larger than the micro-metastasis shown in the H&E section. We have similarly acquired anatomical MR images of cæca of early and highly invasive tumor-bearing mice. However, we have not yet identified any tumors in the lung. The results from DCEMRI showed the contrast uptake in tumors following injection of Gd-DTPA. The two-compartment model was used to analyze dynamic data over the tumor and muscle ROIs to obtain the rate constant for contrast media uptake (K_{trans}) and the distribution of the contrast agent per unit volume of tissue (v_e). The values of K_{trans} (in min^{-1}) and v_e for muscle were found to be of 0.085 ± 0.015 and 0.188 ± 0.025, respectively, similar to those found in the literature. Values of K^{trans} (0.237 ± 0.029 min^{-1}) and v_e (0.395 ± 0.048) for tumors either in cecum or liver were significantly higher ($p<0.0004$) than values for muscle. MR images of tumors in the cecum and liver were also correlated with *in vitro* 2-D histological images.

Discussion: Based on our results with control and tumor-bearing immunotolerant *Rag1* mice, we have demonstrated—for the first time—that *in vivo* anatomical and functional MRI studies, together with *in vitro* histological studies, offer the potential for detecting early colonic cancer invasion and distant metastasis in a murine model, thus making it possible to non-invasively detect cancer in its very early stages. [Supported by grants from NIH (NCI-CA36745), ACS Illinois Division (#08-45), University of Chicago Cancer Research Center, and the Lynn S. Florsheim MRIS facility]