Evaluation of Vanadium-based Contrast Agents for Detection of Early Murine Colon Cancer Using MRI, X-ray Fluorescence Microscopy and a Novel Method of Image Co-registrations

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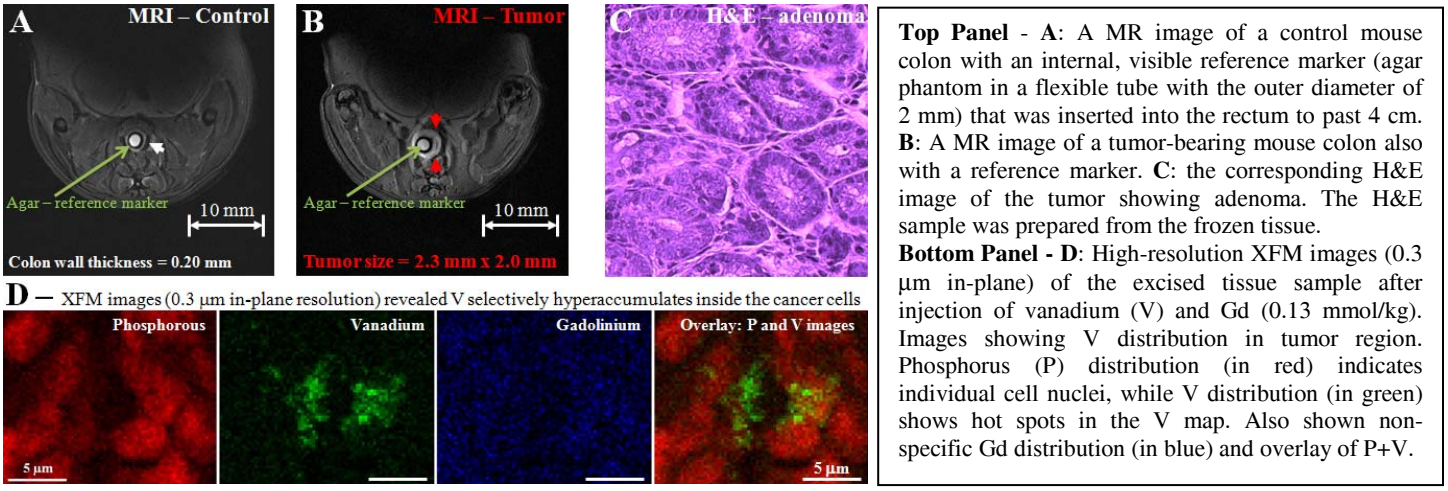
Purpose: Early detection of colon cancer can vastly improve outcomes. Targeted contrast agents that specifically enhance early cancers could significantly improve diagnostic accuracy. Here we compare a new MRI contrast agent that is sensitive to glycolysis to a conventional Gd-based agent. Specifically, we report on: (1) MRI using Gd and a cancer specific, vanadium-based agent (VC) to measure contrast improvement *in vivo*, (2) a novel method of co-registration of *in vivo* and *ex vivo* images using agar-based phantoms, and (3) X-ray fluorescence microscopy (XFM) to quantify contrast uptake directly and to determine cellular and sub-cellular distributions *in situ*.

Methods: Colonic tumors were induced in CF1 female mice (n=25) with i.p. injection of azoxymethane weekly for 2 weeks (10 mg/kg), followed by 2 cycles of 2.5% dextran sulfate sodium in the drinking water for 5 days. This model mimics many clinical and pathological features of colitis-associated colon cancer. T_1/T_2-weighted and contrast-enhanced MR images were acquired using a 9.4 Tesla Bruker scanner. A flexible tube (2 mm o.d.) composed of five 4-5 mm-segments of color-coded agar containing different Gd concentrations was inserted into the rectum, extending up to the cecum. Immediately after sacrifice at the end of *in vivo* MRI experiments, the colons was excised with the fiducial marker in place. This agar-based phantom provided a visible fiducial marker on *in* vivo MR images and served as a ruler for precise co-registration of pathological features detected on MR images with histological morphology. The day after the last *in vivo* MRI, mice were sacrificed after Gd and/or VC injection I.V. (0.13 mmol/kg), colons (normal and tumors) were harvested and ~5 µm thick slices were sectioned for XFM and H&E. XFM images (0.3 - 10 micron in-plane resolution) were acquired using an X-ray microprobe at the Advanced Photon Source at the Argonne National Lab. Concentrations of metal ions and other elements were determined based on the tissue thickness on the slide.

Results: Locations of tumors along the colon were precisely determined on the basis of ager-based, visible fiducial marker, as seen in the figure below. Values of T_2 distinguished normal colon from colonic wall focally thickened with tumor ($p<0.005$). From DCEMRI, the values of K^{trans} (min^{-1}) were found to be 0.12±0.01 for normal colon and 0.61±0.05 for tumors ($p<0.001$). For VC uptake in implanted rodent tumors, relative peak enhancements in tumor and muscle of 0.225 ± 0.052 and 0.05 ± 0.018, respectively, were measured during the injection phase - a nearly 5-fold increase in enhancement between tumor and muscle with $p < 0.001$. VC enhanced specific regions of tumors selectively in MR images, with enhancements typically greater than 50% and often as high as 100%, while no enhancement for Gd. XFM studies of normal and colon cancer-bearing mice suggest specific uptake of VC in tumor regions, while Gd distributes uniformly, as seen in the figure below. High-resolution (0.3 micron) scans of tumors revealed that VC accumulates in the intracellular space in cancers; the uptake of VC in cancer cells is ~8-fold higher compared to the background VC signal.



Top Panel - **A**: A MR image of a control mouse colon with an internal, visible reference marker (agar phantom in a flexible tube with the outer diameter of 2 mm) that was inserted into the rectum to past 4 cm. **B**: A MR image of a tumor-bearing mouse colon also with a reference marker. **C**: the corresponding H&E image of the tumor showing adenoma. The H&E sample was prepared from the frozen tissue.

Bottom Panel - D: High-resolution XFM images (0.3 µm in-plane) of the excised tissue sample after injection of vanadium (V) and Gd (0.13 mmol/kg). Images showing V distribution in tumor region. Phosphorus (P) distribution (in red) indicates individual cell nuclei, while V distribution (in green) shows hot spots in the V map. Also shown non-specific Gd distribution (in blue) and overlay of P+V.

Conclusions: There are potential advantages of using VC-based contrast agents. These compounds have very low toxicity and thus could be used repeatedly at concentrations that produces strong MRI contrast. VC-based agents preferentially accumulate inside of cancer cells, offering an advantage over less selective Gd-based agents. MRI and XFM studies of early colon cancer in mice may improve early detection strategies, provide increased understanding of cancer progression and improve assessment of therapeutic responses.