

In vivo imaging using a VEGF-based probe for early cancer diagnosis in the AOM-treated mouse model

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INTRODUCTION

Angiogenesis is a hallmark of carcinogenesis. Without strong supporting vasculature, tumor cells cannot obtain the nutrients they need to survive and proliferate. Angiogenesis is facilitated by the production of vascular endothelial growth factor (VEGF) which binds to receptors on nearby blood vessels, facilitating blood vessel growth. By detecting and monitoring VEGF and VEGFR, we can improve clinical diagnosis, bolster the efficacy of current treatments, and develop new chemopreventive agents.

In this study, a dual-modality, optical coherence tomography (OCT) / laser-induced fluorescence (LIF) endoscopic system is used to nondestructively evaluate tumor morphology, size, and VEGFR expression in the mouse colon. OCT is an imaging method similar to ultrasound that employs near-infrared light to acquire depth-resolved images with micrometer resolution. However, OCT is only practical for a small field of view, limiting its application to human cancer screening. LIF imaging is used in this study to detect VEGFR, with the *in vivo* application of a VEGF-based, near-infrared fluorescent probe. LIF is already in use to a limited extent in human colonoscopies, using naturally fluorescing tissues, such as collagen, to distinguish normal from diseased areas. This autofluorescence generally has limited contrast and specificity, which may be overcome by using a targeted probe.

In previous work, our lab has demonstrated the efficacy of OCT for detecting tumors in a mouse model *in vivo*, with 95% sensitivity. The VEGF-based fluorescent probe was developed and studied by our collaborators, who noted highly specific uptake of the probe in xenograft tumors. This study is a continuation of this work by studying the efficacy of this probe in a colorectal cancer model that is translatable to human colonoscopy screening. Mice used in this study were treated with a carcinogen, azoxymethane (AOM), which causes tumors to grow spontaneously in the lower colon. A miniature endoscope is used to nondestructively collect longitudinal OCT/LIF images. Using criteria developed in our lab for identifying tumors in OCT images, we were able to map adenomatous regions in the colon and compare this data with co-registered fluorescence data from the VEGF-based probe.

MATERIALS AND METHODS

The VEGF-based probe, single chain (sc) VEGF/Cy5.5, was prepared by our collaborators. Briefly, two fragments of human VEGF₁₂₁ were cloned head-to-tail and Cys-tagged with a fluorescent dye, Cy5.5 maleimide. This dye was chosen for its red excitation and red/infrared fluorescence, wavelengths that are safe for human use, can penetrate more than a millimeter in tissue, and have minimal overlap with tissue autofluorescence.

Mice were treated with a carcinogen, azoxymethane (AOM), with five weekly intraperitoneal injections 16-23 weeks prior to imaging. Two methods of administering the fluorescence probe were tested and

compared. First, six AOM-treated mice received scVEGF/Cy5.5 via intravenous (IV) injection in the tail vein. Then, seven AOM-treated mice and two control mice received scVEGF/Cy5.5 via colon lavage. Also, four AOM-treated mice and two control mice received a scrambled dye, inactive (in) VEGF/Cy5.5, via colon lavage.

A dual-modality OCT/LIF endoscope was lubricated and inserted in the colon. For each mouse, eight longitudinal OCT and LIF images were collected in 45 degree intervals around the colon. After imaging, the animals were sacrificed and the colons were explanted. Colons were prepared using standard paraffin embedding technique and sections were stained with H&E.

The eight OCT images were used to map adenomatous regions in the colon. These adenomas were verified with H&E stained histological sections. To compare the fluorescence data with detected tumors in OCT, the images were chunked into 5 millimeter segments and labeled as either diseased or normal. The maximum Cy5.5 fluorescence intensity in each corresponding segment of the LIF images was determined. A sliding threshold for Cy5.5 fluorescence intensity was used to calculate sensitivity and specificity for disease.

RESULTS AND DISCUSSION

The data for IV injection and colon lavage of scVEGF/Cy5.5 were considered separately. A number of confounders were observed for IV injection, such as the small intestine, mammary fat pad, and bladder. These tissues consistently produced a large fluorescence signal that often masked the fluorescence from tumors. Lavage administration eliminated these confounders to a large extent and allowed fluorescence from tumors in the colon to dominate. We found high variability in fluorescence between tumors, possibly due to differences in developing vasculature. Receiver-operator characteristic curves for both methods are shown in Figure 1. Despite high variability in the fluorescence between tumors noted for the lavage method, the sensitivity/specificity is still high, 83%/85% for one threshold.

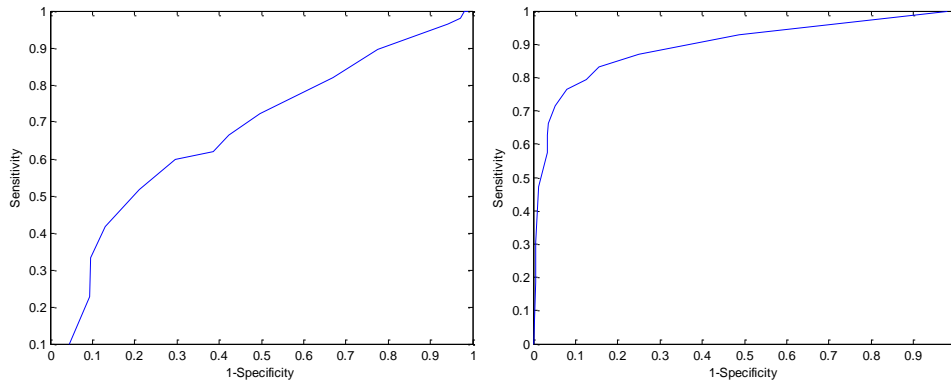


Fig. 1: Sensitivity versus (1-Specificity) is plotted for various fluorescence value thresholds for both the IV (left) and lavage (right) administration methods. Note the improved performance with the lavage method.

Fluorescence from Cy5.5 in control mice, which received scVEGF/Cy5.5 via colon lavage, was markedly less than that in the AOM mice. Two sample OCT/LIF image pairs are shown below for both an AOM mouse and control mouse. Quantitatively, the Cy5.5 fluorescence in the control mice did not exceed a

value of 0.02 arb. units; whereas, the threshold resulting in 83%/85% sensitivity/specificity was 0.06 arb. units, three times the maximum value in the control mice.

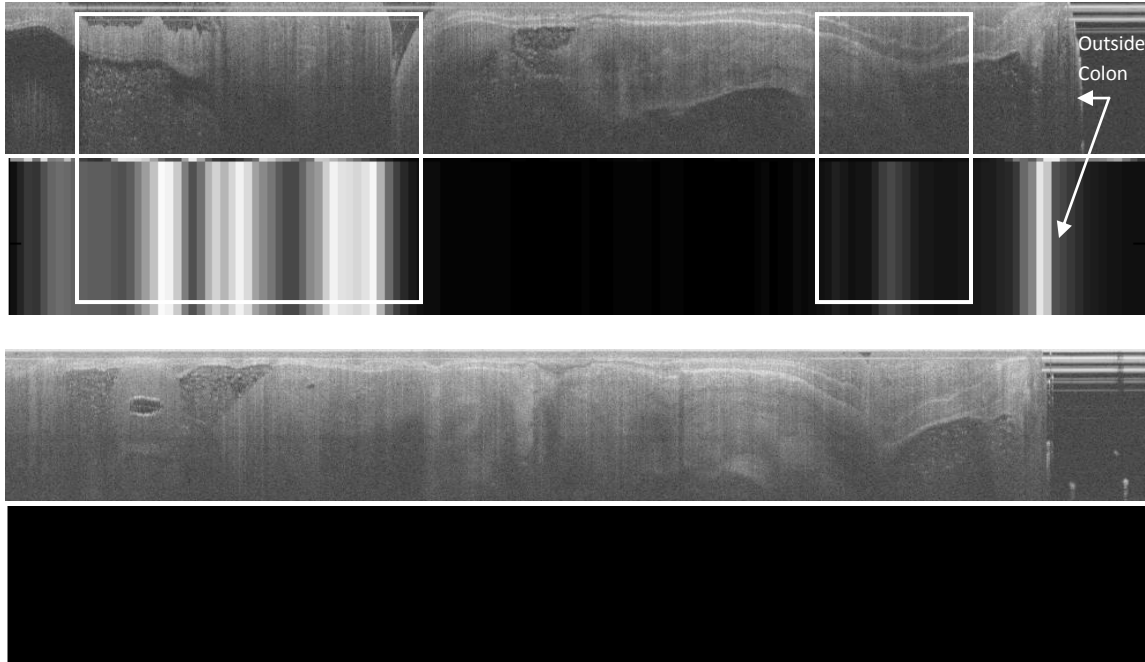


Fig. 2: OCT/LIF images for an AOM-treated mouse (top) and control mouse (bottom). The AOM-treated mouse exhibits a highly fluorescing proximal tumor on the left-hand side and a slightly fluorescent developing distal tumor on the right-hand side (white box). The anal region also exhibits some fluorescence (arrow), probably due to dye caught in the fur. By contrast, little fluorescence can be noted in the control mouse.

To test how well fluorescence from the dye correlates with VEGF-receptor expression in our model, we are planning a short, follow-up study on explanted colon tissue. We will compare localization of dye fluorescence from a thin histological section, exposed to scVEGF/Cy5.5, with adjacent (6 μm spacing) histological sections immunostained for VEGF-receptors 1 and 2.

Colon lavage from the scrambled probe, inVEGF/Cy5.5, resulted in patterns of fluorescence emission that provide similarly high sensitivity/specificity for tumors, 87%/85% for one threshold (0.125 arb. units). Fluorescence microscopy images *ex vivo* revealed that inVEGF/Cy5.5 gathered in the many folds in tumors, enhancing the more convoluted tumor tissue more than the smooth normal tissue. The *ex vivo* images of scVEGF/Cy5.5 showed clumped and heterogeneous uptake in tumors, presumably associated with VEGFR distribution. Due to limitations of the AOM-treated mouse model, it is uncertain as to whether inVEGF/Cy5.5 or scVEGF/Cy5.5 would be able to highlight flat, occult regions, often missed in human colonoscopy screening. Future studies in other models or in early, less pronounced disease will be necessary to test the ability of the agents to detect these lesions.