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Current and Future Imaging Paradigms in Colorectal Cancer

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Introduction

Imaging technologies have rapidly advanced over the past few decades and currently play a central role in colorectal cancer patient management. Marked advances in anatomic imaging (MRI, CT - including virtual colonoscopy - and endoscopy) continue to improve tissue contrast, spatial resolution, and acquisition speed, allowing higher rates of lesion detection, improved accuracy, and decreased patient morbidity. However, augmentation of such anatomic imaging with the superimposition of spatially resolved molecular information from the observed tissues fundamentally changes how we view imaging. The field of molecular imaging, which focuses on evaluation of levels of biologically important molecules, pathways, and activities presents a new paradigm in disease management.

Molecular imaging has been formally defined as the detection, spatial localization, and quantification of specific molecular targets and events that form the basis of various pathologies (1). Bench-side [TERM? “Basic” better?]? Laboratory research in molecular imaging has flourished due to complementary advances in the understanding of cancer pathways, imaging ligand design, and novel imaging hardware development. By detecting disease states and response to therapy as changes in the level and function of molecular targets, earlier evaluation is possible than that possible through the evaluation of anatomic changes that are a downstream result of these molecular alterations. Numerous targets that influence tumor behavior and response to therapy, ranging from cell surface receptors, tyrosine kinases, proliferation markers, proteolytic enzymes, extracellular matrix targets, apoptotic markers, angiogenesis markers, and glucose metabolism levels, among many others, have been evaluated for non-invasive imaging. This information may improve patient care by finding additional lesions which cannot be detected with traditional anatomic imaging modalities. Once disease is found, molecular imaging may play a vital role in individualizing treatment by aiding in selection of appropriate molecularly-targeted drugs and in accurate assessment of adequate drug dosing. Finally, evaluation of metastatic disease burden, especially early lymph node involvement, may be improved by such functional (metabolic) assessment. Molecular imaging has already recently expanded from preclinical to clinical trials and in some cases to routine clinical use. The goal of this review is to highlight several of the advances that are particularly relevant in the context of colorectal malignancy.

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Current State of the Art

Direct visualization of colorectal cancer and precancerous adenomas by minimally invasive colonoscopy has been employed over decades, is a mainstay of early detection today through screening programs (2) and permits immediate treatment such as polypectomy where applicable. Colonoscopy intrinsically provides high spatial resolution images of anatomic aberrations ranging from subtle alterations in mucosal patterns to gross luminal narrowing. Traditional fiber-optic based endoscopes have been largely supplanted by microchip cameras able to fit onto the tips of catheters (3), thereby further improving image resolution and clarity. Despite these engineering advancements, adenoma detection has ultimately been shown to be largely operator- and time-dependent (4) given the subtlety of detecting early lesions across a large organ surface. Attempts have been made to address some of the shortcomings of colonoscopy, such as development of microchip cameras engineered to fit entirely within a capsule. Such systems are ingested by the patient, image snapshots of the entire gastrointestinal tract during their passage, and are particularly helpful in the detection of small bowel pathology. However, during colonic evaluation the stochastic time and location of each image acquisition has been shown to miss a number of prominent lesions (5). All detection schemes based on visible wavelength, including traditional endoscopy, have been limited to the detection of primary intestinal lesions, and are not useful for determining the presence of metastatic disease outside of the colonic lumen. Whole body tomographic imaging modalities provide complimentary imaging information for such evaluation.

Radiologic cross-sectional imaging methods such as MRI and CT, similar to endoscopy, mostly rely on anatomic distortion in the size and shape of normal structures or the creation of luminal “filling defects” to detect primary tumors. The alterations may be further enhanced by temporal perfusion differences between tumor and normal organ parenchyma highlighted by the use of non-specific small molecules such as iodinated contrast agents and Gd-DTPA (6). These techniques have excelled at finding metastatic foci, for example colonic metastases to the liver, and their use for such applications is part of the routine standard of care. Their role in evaluation of the primary site of disease has been less well defined. For example, MRI using phased array or endorectal radiofrequency coils has been evaluated for detection of muscularis propria invasion (7,8). Over the last few years, virtual CT scanning has gained an important role in the screening of patients for polyps and colonic cancers (9). Again, identification of primary sites of disease, similar to detection of metastatic foci, is determined by physical alterations in expected anatomic shape and patterns.

A combination of molecular activity mapping and anatomy has been rapidly growing in clinical practice over the last five years with the increased use of PET-CT imaging (10). While many of the fundamental processes that underlie cancer have pathways or molecules that may be altered in disease and have radiolabeled imaging analogs that allow their visualization (11), the overwhelming majority of clinical PET and PET-CT scans utilize ^{18}F -fluorodeoxyglucose (FDG). Areas of increased glucose utilization are highlighted with this methodology and are extremely valuable for tumor staging, especially for the detection of metastatic foci. Studies have made initial forays into evaluation of FDG PET imaging for detection of primary colonic neoplasms in a screening setting (12) and have also preliminarily looked at PET-CT for accuracy of staging (13). However, a number of important caveats exist for such use. Whereas glucose utilization is increased in many tumor types and this molecular information complements the anatomic changes seen by CT, FDG PET imaging has been shown to have relatively poor sensitivity in detecting some tumor types, for example mucinous adenocarcinomas. This may be due the relative hypocellularity of these lesions, as studies have show inverse correlation of FDG PET detectability with mucin content and direct correlation with tumor cellularity of these cancers (14,15). Additionally, detection threshold for small lesions, such as lymph node metastases, depends upon metabolic activity of the tumor foci.

Sensitivity is particularly variable for metastatic foci in lymph nodes that measure less than 1 cm in short axis dimension.

FDG PET and FDG PET-CT are part of the current state of the art clinical practice in tumor staging. However, several newer molecular imaging techniques and applications are currently on the near term horizon for colorectal cancer detection and evaluation in patients. In the same vein as FDG PET imaging, these technologies represent new ways to visualize biological processes on a molecular scale. They complement not only current anatomic methods, but in many ways complement the information routine FDG PET scanning provides. One set of techniques focuses on an iron oxide nanoparticle used for a range of applications in MR imaging. The other technology revolves around the use of functionalized fluorochromes, including smart agents, which enable new methods in optical imaging.

Magnetic Iron Oxide Nanoparticles

An area of particular interest in tumor staging is the determination of metastatic tumor cells within draining lymph nodes, since this has direct implications not only for surgical resection planning but also for possible post-resection radiotherapy. The current standard of care for determination of lymph node metastases based on non-invasive imaging criteria involves predefined normal sizes for various lymph node groups. For many nodal groups, a short axis diameter of greater 1 cm in elongated lymph nodes or greater than 8 mm in round lymph nodes is considered indicative of malignancy, while those nodes that do not meet this criteria are considered benign (16). However, lymph nodes can vary considerably in size due to inflammation and other benign causes, resulting in poor accuracy of determining metastatic status based on lymph node size alone. One study that evaluated size criteria for lymph node metastases from rectal cancer demonstrated that using a 5 mm size cut off (that is, the presence of lymph nodes greater than 5 mm were considered a sign of malignant involvement), the sensitivity and specificity based on size alone was only 73 and 75%, respectively (17). Thus, a molecular imaging adjunct to determination of anatomic changes could help fulfill a useful role in evaluation of lymph node metastases.

Magnetic nanoparticles were first evaluated in experimental systems for enhancement of MR imaging in the late 1980's. In addition to physiological and molecular specificities conferred by their size and surface coating, improved detection has been a major motivating force in their use: the typical detection threshold of traditional contrast agents such as Gd-DTPA is approximately 10^{-4} M, whereas iron oxide agents have a detection threshold in the 10^{-8} M range. These nanoparticles usually have a superparamagnetic core of iron oxide with a coating that changes the biodistribution and blood half life of the particle. A commonly used coating is dextran sulfate, but other coatings including citrate have been evaluated. Based upon particle size, nanoparticles have been classified as SPIO (small particle iron oxide) or USPIO (ultrasmall particle iron oxide). While SPIO agents such as Feridex have been approved for use for the detection of hepatic metastases by MRI, imaging technologies using standard small molecule contrast and anatomic imaging have been so effective that this is currently a minor application for Feridex use. A growing use in experimental systems and likely clinical application in the future is the use of Feridex for cell tracking (18).

During evaluation of SPIO particles, a longer circulating, smaller particle size fraction was discovered. This led to the development of USPIO's. The use of USPIO agents for lymph node imaging was first evaluated in 1990 (19,20). USPIO monocrystalline iron oxide core size is approximately 4 nm in diameter, with the dextran coating resulting a shell that has a diameter of approximately 40 nm. Long circulation times are accomplished because the small size of the particles delays rapid hepatic or renal clearance. The last decade has seen an expanding growth in the clinical evaluation of the USPIO Combidex (21,22). When intravenously

administered, Combidex remains initially within the vasculature. Over time, the particle slowly extravasates into the extracellular space and is cleared by lymphatic drainage. Resident macrophages within the lymph nodes phagocytize the particles. This uptake and subsequent subcellular localization result in local magnetic field changes that may be detected by T2 and T2* weighted MR imaging. Small tumor metastases to lymph nodes may alter drainage and nanoparticle uptake in several ways, including exclusion of macrophages in areas of tumor, obstruction of afferent or efferent lymphatic channels, or alterations in lymphatic macrophage function. In a large clinical trial evaluating detection of prostate lymph node metastases, the sensitivity and specificity of the detection by MRI alone for the important size range of 5–10 mm diameter lymph nodes was 28.5 and 87.2%, respectively, whereas this increased to 96.4 and 99.3%, respectively, based upon MR imaging in combination with Combidex administration (23).

An important aspect of this paradigm is that image contrast depends upon host response. Thus, the method has wide applicability across cancer types. USPIO evaluation of lymph node metastases is complementary to FDG PET imaging in several important ways. First, the method allows detection of metastases irrespective of the metabolic state of the tumor and is thus much less dependent upon the primary tumor type. Clinical trials are underway to image lymph node metastasis in tumors as diverse as breast, testicular, prostate, lung, and pancreatic cancer. Second, small metastatic foci are detectable based on sub-lymph node drainage and uptake patterns, whereas PET lymph node metastasis imaging is based upon a global metabolic activity within the entire lymph node, in part due to differences in typical voxel size between the techniques. As seen in Fig. 1, agent uptake can be used to help delineate rectal cancer metastases in local lymph nodes. The same method may also be applied to image mesenteric lymph nodes from more proximal colonic lesions.

Understanding the physiological clearance of the USPIO's allows determination of other useful parameters that help define tumors and treatment response. Early imaging (within the first few hours after intravenous injection of the nanoparticle), allows the calculation of the percent fraction of tumor containing a functional microcirculation. Based upon modeling of microscopic magnetic field changes with USPIO's in the microvasculature, comparison of MR T2* imaging sequences before and after nanoparticle administration allow determination of vascular volume fraction (VVF) (24). As seen in Fig. 2, the change in T2* allows spatial variations in VVF to be evaluated across a tumor on a pixel by pixel basis. In colorectal cancer, anti-angiogenic therapy is being explored for efficacy when combined with a number of other therapy combinations (25). Thus, the same nanoparticle allows early imaging of vascular changes with therapy, whereas delayed imaging, performed at 24 hrs post IV administration, allows detection of possible lymph node metastases. Similar approaches to imaging the tumor microvasculature using other macromolecular MR agents have been shown in experimental systems to determine responsiveness of colorectal tumors to anti-angiogenic therapy (26).

Near Infrared Optical Imaging Probes

In a study utilizing back-to-back colonoscopies more than 20% of colonic polyps were missed (27). Such anatomic imaging is even more likely to miss flat lesions which may have higher rates of dysplasia compared to polyps (28). Direct visualization of adenomas and colorectal cancer by endoscopic methods remains the clinical standard, but engineering improvements of the white light imaging paradigm, by themselves, are unlikely to resolve the missed lesion rate. This problem is further exacerbated in ulcerative colitis (UC), in which dysplasia can develop in macroscopically normal-appearing mucosa (29). Current colonoscopic surveillance in UC patients relies on random biopsies throughout the colon, which is relatively insensitive and cumbersome (30). In the case of intraperitoneal spread of cancer, detection of small

metastatic foci intraoperatively may be limited by the similar luminosity of tumors compared to adjacent normal tissue.

These clinical needs may be addressed in part using a novel approach that combines new imaging devices and fluorescent imaging probes. The near infrared (NIR) region of the electromagnetic spectrum is immediately adjacent to the visible spectrum, which extends from violet to red. NIR imaging offers a number of important advantages. There is less autofluorescence in the NIR compared to visible wavelengths; decreased background improves visibility (the target to background ratio) of exogenously administered fluorochromes. It is also possible to split light across different wavelengths, similar to a prism. This allows true simultaneous imaging of the full spectrum of visible light, and NIR light. The anatomic view that surgeons are currently accustomed to using is maintained, while a second camera records fluorescence that is generated by molecular reporters. A clinical NIR system has been approved for evaluation of coronary artery bypass graft patency using the NIR fluorochrome indocyanine green (31). Alternatively, the system's field of view potentially allows for intraoperative abdominal viewing. We have developed clinically translatable fluorescent micro-endoscopes that allow luminal viewing and realtime quantitative analysis of fluorescent signal during colonoscopy or laparoscopy (32). Such devices augment standard anatomic white light imaging of the colon and peritoneum with simultaneously registered NIR imaging.

The other vital components to molecular optical imaging are the imaging agents. There are three general classes of imaging probes: non-specific agents, targeted agents, and smart probes; fluorescent versions of all three have been used extensively in preclinical studies. The smart probes have been particularly promising for lesion detection. This class of optical imaging agents increases fluorescence after interacting with specific proteolytic enzymes, such as cathepsins (33). These imaging agents (diagramed in Fig. 3) are initially optically silent due to fluorochrome-fluorochrome quenching when the fluorochromes are in close proximity, but become brightly fluorescent in areas of protease overexpression seen in a variety of disease states. Cleavage results in marked signal amplification, yielding very high target to background ratios during fluorescence imaging that maps protease expression. Cathepsins have a number of advantages as imaging targets. They have been heavily implicated in cancer invasion, metastasis, and proliferation (34). Additionally, many tumor types and the host tissue response to tumors both result in increased signal, making the approach generalizable. Additionally, a broad range of agents sensitive to a diverse array of proteolytic targets have been developed, including matrix metalloproteinases (35) and other cathepsins (36), allowing for additional in vivo characterization.

Imaging of cathepsin protease activity has resulted in improved detection of neoplasia in preclinical models including models of ovarian cancer (32) and transitional cell carcinoma of the bladder. Target to background ratios as high as 9:1 have been observed in peripheral lung cancer when using NIR light and protease sensitive probes, compared to a target to background ratio of approximately 1:1 for standard white light thoracoscopy (37). This approach was also tested for imaging of murine models of intestinal adenomas (38), and demonstrated improved ex vivo detection of non-invasive polyps as small as 50 micrometer in diameter. NIR light can be separated into several distinct wavelength bands that can independently record distinct molecular activities, each reported by different fluorochromes (39,40). As seen in Fig. 4, we have applied this multi-parameter approach to evaluate colonic adenomas and adenocarcinomas in terms of protease activity and lesion perfusion, to help non-invasively segregate lesions between these groups. The protease imaging agents and endoscopic systems are both clinically translatable, and clinical trials for colonoscopy are expected to begin in the next year or two. Initial preclinical work suggests that the approach may also be effective in detecting adenocarcinomas in the setting of colitis, based on differential protease expression between the sites of neoplasia and inflammation.

Apart from protease-activatable probes, there are a number of other targeted and non-specific optical probes applicable to the detection and therapy management of colorectal cancer. One notable example is the optical analog to the intravascular MRI agents discussed previously. These compounds are fluorescently labeled macromolecules (41) that maintain a long blood half-life. Such fluorescent blood pool agents are especially useful for determining the vascular volume fraction of known tumors. This type of imaging has been shown in pre-clinical models to non-invasively assess vascular changes after anti-angiogenic therapy. In a future clinical setting, a similar approach may be employed to evaluate treatment efficacy of drugs such as bevacizumab, recently approved for metastatic colon cancer (42). As seen in Fig. 5, such evaluation may be performed during fluorescent colonoscopies. Radiolabeled targeted imaging agents have been extensively evaluated by the nuclear medicine community. Analogous optical agents have been generated for a number of important targets. Additionally, optical agents have also been made for new targets in abdominal imaging. Fluorescent somatostatin analogs (43, 44), fluorescent moieties that bind various integrins (45,46), and fluorescent folate analogs (47,48) have all been evaluated for improved in vivo imaging of abdominal neoplasia in preclinical models.

Conclusions

With the adoption of FDG PET imaging into routine clinical practice, the concept of molecular imaging technology has already been introduced into mainstream use and has altered cancer staging workup. However, specialized new MRI based and optical imaging techniques in combination with exciting new imaging agents have undergone extensive preclinical, and in some cases clinical, testing, and are now on the horizon for expanded human use. In particular, MRI with iron oxide nanoparticles and fluorescent endoscopy and intraoperative imaging with activatable optical smart probes are two near term future imaging paradigms. Given the profound effect these developments may have on cancer diagnosis and prognosis, it is worthwhile for all clinicians, including colorectal surgeons, to pay close attention to ongoing clinical trials and advanced preclinical results.

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Figure 1.

T2* weighted MR images of the pelvis from a patient with rectal cancer injected intravenously with the iron oxide based nanoparticle Combidex one day prior to imaging. Benign lymph node (white arrow) demonstrates uniform uptake and darkening. Two sub-centimeter lymph nodes (black arrows) demonstrate heterogeneous uptake (panel A) and no uptake (panel B) due to lymph node metastases. Images courtesy of Mukesh Harisinghani, MD.

Figure 2.

A. T1 weighted image from a mouse with a subcutaneous tumor. B. False color T2 map superimposed over the tumor. Images in the blood pool phase were acquired immediately post injection of MION, a dextran coated iron oxide nanoparticle. Pre- and post-injection differences allow calculation of vascular volume fraction on a pixel by pixel basis within the tumor. Such an approach is useful for non-invasively monitoring anti-angiogenic therapy.

Figure 3. Schematic diagram of smart optical probe activation after protease cleavage. Fluorochromes (black circles) are covalently coupled to a protected poly-lysine backbone. Due to the proximity of the fluorochromes, auto-quenching occurs so that almost no fluorescent signal can be detected in the non-activated state. After designated enzyme cleavage of a peptide spacer (green), fluorochromes are released from the carrier and become brightly fluorescent (red circles).

Figure 4.

A series of murine colonoscopies demonstrating (from top to bottom): normal colon, adenomas, and adenocarcinomas. Full color white light images were acquired simultaneously with two near infrared channels, each reporting upon different molecular-activity fluorescent probes. A normalized ratio of protease activity, measured in one NIR channel, divided by perfusion, imaged in a second NIR channel, increases from normal colon to dysplastic adenomas to invasive adenocarcinoma. Protease activity imaging brightly highlights both adenomas and adenocarcinomas, aiding in their detection. Reprinted with permission from *Gastrointestinal Endoscopy*, 2006;64:589–97.

Figure 5. White light (A, C) and near-infrared visualization of a fluorescent blood pool agent (B, D) from a normal murine colonic segment (top) and one with adenomas projecting into the lumen (bottom). Fluorescent signal intensity correlates with lesion microvascularity and may be used for evaluation of anti-angiogenic therapy. Note blood pool retention is readily visualized in larger vessels (A, B).