Molecular endoscopy for targeted imaging in the digestive tract

Jeong Hoon Lee, Thomas D Wang

Endoscopy uses optical imaging methods to investigate tissue in a non-destructive manner with high resolution over a broad range of wavelengths, thus providing a powerful tool to rapidly visualise mucosal surfaces in the digestive tract. Molecular imaging is an important advancement that has been clinically demonstrated for early cancer detection and guidance of therapy. With this approach, imaging can be used to observe expression patterns of molecular targets to improve understanding of key biological mechanisms that drive disease progression. Prototype devices that collect fluorescence for wide-field or microscopic images have been developed. Several targeting moieties, including enzyme-activatable probes, antibodies, peptides, and lectins, have been administered in preclinical and clinical imaging studies in vivo. These emerging technologies provide useful approaches to study molecular events in different signalling pathways, producing insights that could lead to improved interventions to prevent and treat gastrointestinal diseases. In this Review, we introduce the basic concepts that form the foundation for development of molecular endoscopy and summarise key results from preclinical and clinical studies.

Lancet Gastroenterol Hepatol 2016; 1: 147–55

Department of Gastroenterology, University of Ulsan College of Medicine, Asan Medical Center, Seoul, South Korea (J H Lee MD)**; and Department of Internal Medicine, Division of Gastroenterology, Department of Biomedical Engineering, and Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI, USA** (Prof T D Wang PhD)

Correspondence to:
Prof Thomas D Wang, Division of Gastroenterology, University of Michigan, Ann Arbor, MI 48109, USA
thomaswa@umich.edu

Introduction

Endoscopy uses human orifices to directly visualise large epithelial surfaces in hollow organs.[1] This minimally invasive procedure has undergone rapid growth in sophistication, meaning that every region of the digestive tract can be accessed easily and visualised with great clarity. Grossly visible architectural abnormalities, such as polyps and masses, and discolourations, such as erythema, can aid detection of the presence of disease. Molecular imaging is an emerging technique that has been adapted for use as an adjunct during endoscopy to visualise biological phenomena,[2] and promises to provide exciting new opportunities for early cancer detection, guidance of tissue biopsy sampling, and determination of therapy choice. These approaches can be harnessed to visualise genetic and molecular alterations that drive disease processes, and thus have great potential to advance the practice of medicine. Advanced imaging methods—eg, chromoendoscopy,[3] narrow-band imaging (NBI),[4] and autofluorescence imaging (AFI)[5]—have been clinically evaluated using non-specific contrast mechanisms, such as vascular and mucosal patterns. Molecular endoscopy offers the opportunity to substantially improve specificity through detection of targets that are unique to disease. This technique has been demonstrated in preclinical models using genetically engineered animals that replicate the molecular pathogenesis of human disease.[6] Molecular endoscopy has been demonstrated clinically in the colon[7] and oesophagus,[8] and is being developed for use in the stomach, biliary tract, and pancreatic duct. In this Review, we introduce basic concepts that serve as the foundation for development of molecular endoscopy—including optical properties of tissue, specifications of devices, and characteristics of probes—and summarise key results from preclinical and clinical studies done in vivo.

Clinical need for improved imaging

Precancerous lesions in the digestive tract can be flat in architecture and focal or patchy in distribution, making them difficult to detect with conventional white light endoscopy.[9] The effectiveness of cancer surveillance could be improved if the detection sensitivity were increased through strengthening of the contrast between lesions and the surrounding tissue. The existing standard for diagnostic decision making is based on observation of structural changes and identification of anatomical landmarks. Visualisation of biological properties and imaging of molecular expression helps to detect the presence of flat lesions. Detection specificity can be improved through imaging of overexpression of cell surface and intracellular targets that are unique to disease, thus reducing overdiagnosis.[10] The information collected can be used to understand molecular mechanisms that drive disease progression and to predict the likelihood for cancer progression and individual response to therapy. This strategy can result in the development of new methods for early cancer detection, personalised therapy, and chemoprevention. Overall, molecular imaging promises to substantially broaden the capability of the diagnostic tools available to gastroenterologists by provision of new approaches to visualise digestive tract mucosal biology.

Molecular imaging has its origins in radiology, in which the presence of somatostatin receptors on the cell membrane of neuroendocrine tumours was first detected with scintigraphy using radiolabelled octreotide.[11] This approach has been adapted for use with PET, which has become the preferred imaging modality for staging of advanced cancer.[12] Despite nuclear medicine techniques having advantages for deep organ penetration, optical imaging methods have greater promise for early cancer detection because neoplasia originates within the epithelium,

and mucosal surfaces in the digestive tract and other hollow organs can be rapidly and thoroughly inspected.[13] Additionally, endoscopy can facilitate accurate diagnosis with so-called intelligent biopsy sampling to minimise unnecessary physical biopsy sampling and bleeding. Molecular imaging has been introduced in endoscopy, and shows potential to provide enhanced contrast and improved specificity.[14] In the digestive tract, the imaging agent can be administered topically in high concentrations that are safe for patients to achieve high image contrast for disease detection.[15] Because of the broad spectrum of light, optical methods can also be used in a multiplexed configuration in which multiple targets are detected concurrently.[16] This novel approach is useful for detection of genetically heterogeneous lesions. Conventional methods used in radiology cannot match the flexibility of optical imaging for this purpose.

Use of light in endoscopy

Molecular endoscopy has been developed on the basis of fundamental optical properties of tissue (figure 1). Haemoglobin intensely absorbs visible light (400–700 nm). This effect is used by advanced non-molecular methods such as NBI to generate contrast from lesions that are highly vascularised. NBI provides illumination in the blue (440–460 nm) and green (540–560 nm) regions to match the peak absorption of haemoglobin to highlight mucosal capillaries and submucosal veins.[17] AFI uses blue light to excite endogenous fluorescence from collagen, flavins, and porphyrins.[18] Although these methods have shown some promise in the clinic, they cannot distinguish between neoplasia and inflammation, and are limited by high false-positive rates and poor specificity.[19] Despite encouraging clinical studies, various governing societies have not recommended routine use of NBI and similar techniques.

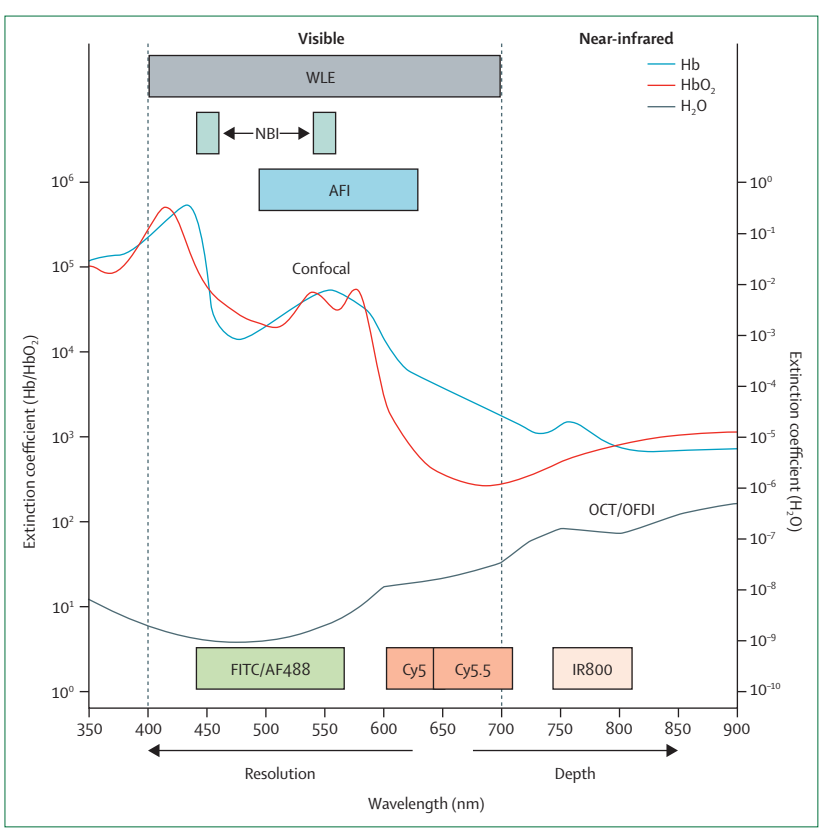

Figure 1: **Optical properties of tissue**
Haemoglobin (Hb) and oxyhaemoglobin (HbO_2) absorption dominates the visible spectrum, used by white light endoscopy (WLE), but has minimal effect in the near-infrared (NIR) range. AFI=autofluorescence imaging. NBI=narrow-band imaging. OCT=optimal coherence tomography. OFDI=optimal frequency domain imaging. FITC=fluorescein isothiocyanate.

Fluorescein is a non-specific visible fluorophore approved by the US Food and Drug Administration (FDA) for human use,[20] and has been used to generate contrast in several studies using confocal endomicroscopy.[21] Fluorescein isothiocyanate (FITC) is an inexpensive derivative that is often used to label molecular probes. These fluorophores emit at short wavelengths in the visible spectrum, and provide high image resolution with reduced depth. Optimal coherence tomography (OCT) and optimal frequency domain imaging (OFDI) use backscatter of near-infrared (NIR) light to achieve deep tissue penetration.[22] Additionally, NIR fluorophores such as Cy5, Cy5.5, and IRDye800, which emit in the 665–900 nm range, can generate fluorescence with similar results. These dyes have been used in clinical studies via an Investigational New Drug application approved by the FDA. Autofluorescence contributes minimal background at long wavelengths.

Targeting of specific contrast agents

Molecular endoscopy exploits the uniqueness and high expression levels of targets that are specific for disease. The ideal imaging agent has a safe toxicity profile, high affinity, specific target uptake, rapid clearance from non-target tissues, good vascular permeability, in-vivo stability, non-immunogenicity, and low cost. The use of exogenous agents in a general patient population could raise safety concerns—depending on method of administration—that might be justified in high-risk subgroups. Systemic delivery provides comprehensive distribution of the imaging agent throughout the entire body, produces deep tissue penetration, and is useful for staging of disease, but can be limited by allergic reactions, toxicity, and biodistribution to non-target tissues. This approach is well suited to organs with large surface areas, such as the colon. Topical administration with use of a spray catheter is practical for detection of focal disease, such as that found in Barrett's oesophagus. This approach can deliver the imaging agent in high concentrations directly to the mucosa at risk, thus minimising safety concerns and achieving a rapid effect.