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Review Article

Molecular imaging in gastroenterology: A route for personalized endoscopy

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ABSTRACT

With the rapid expansion and diversification of the repertoire of biological agents utilized in inflammatory bowel diseases and cancer and the increase in oncological patients in gastroenterology, visualization of single receptor or molecular target expression and the subsequent initiation of expression tailored therapy are gaining increasing attention. Through the combination of utilizing fluorescently labeled probes with high specificity towards defined molecular targets and their subsequent detection and visualization with endoscopic devices, molecular imaging is a new emerging field focusing on the receptor expression within the mucosa on a cellular level rather than on macroscopic changes. In the past years various new technological and molecular probes have been successfully utilized for molecular imaging. Within this review, we summarize different technologies as well as molecular probes applied in molecular imaging and review current and past approaches for functional imaging with molecular endoscopy within the GI Tract and resulting clinical applications. It can be expected that molecular imaging allows for individualized diagnostic approaches and patient tailored medicine in the future.

1. Introduction

White-light endoscopy (WLE) has been established as a gold standard for the detection of preneoplastic or inflammatory conditions in the upper and lower gastrointestinal (GI) tract. However, there is still a high miss rate during WLE even of lesions with advanced histological features [1–3]. Therefore, in the past years technological improvements together with advantages and improvements in development of molecular probes and antibody engineering have led to the rapid expansion of molecular imaging as an approach that integrates basic science and clinical endoscopy. Molecular imaging is a new field in gastroenterology in which fluorescently labeled probes with high specificity towards defined molecular targets are subsequently detected and visualized with endoscopic devices, thereby enabling visualization of single molecules or receptors. Therefore, molecular imaging focuses on the detection of molecular changes rather than on macroscopic appearances [4] and offers the possibility to visualize targets that are specific and unique for certain diseases [5]. After first studies have successfully utilized molecular imaging in precancerous and cancerous lesions as well as in intestinal inflammation in murine models, the past decade has shown that molecular imaging is not only feasible in humans, but can at the same time tremendously expand and enhance detection as well as characterization of lesions and furthermore holds the potential to make individual prediction about the success of subsequent biological therapies. With this, molecular imaging might be the first approach that allows for patient stratification and patient or risk-tailored individual therapeutic decision on the basis of endoscopy.

Within this review, we will start off by describing the different imaging devices as well as the molecular probes used for molecular imaging. Afterwards, we will show examples of clinical applications of molecular imaging throughout the GI-tract and discuss how these might influence and determine clinical decision-making.

2. Methods for molecular imaging

2.1. Technologies

Endoscopic devices utilized for molecular imaging can be generally divided into two categories: macroscopic and microscopic molecular imaging technologies (Table 1). With macroscopic imaging a wield-field endoscopic image is acquired and hence, macroscopic imaging is suitable for lesion detection and can be used as a "red-flag" technique. In contrast, microscopic imaging

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Table 1
Endoscopic devices used for molecular imaging.

Device	Advantages	Disadvantages
Autofluorescence imaging [6,45,46,47,52]	• Wide-field detection, utilization as a red-flag technology	• Low specificity
Near-Infrared imaging [8,55]	• Useful for imaging of molecular targets	• Limited experience in humans
Confocal [9,10,11,42,43,57,58] endomicroscopy/pCLE	• Microscopic imaging with high magnification	• High costs
	• Can be used with any existing endoscopy systems • Allows microscopic video recording	• Limited field of few
Endocytoscopy [11,15]	• Characterization of tissue with high magnification	• High costs
	• Possibility to continuously zoom from standard view to magnification with one scope	• Few clinical trials

is not ideal for screening or detection due to the limited field of view; however, endoscopic devices with microscopic resolution offer detailed characterization at cellular level [4].

Fluorescence spectroscopy is based on the principle that all tissues exhibit endogenous (auto) fluorescence, when illuminated by light of a specific wavelength. Among these, autofluorescence imaging (AFI) has been shown to be a promising macroscopic technique for wide-field endoscopic imaging. Here, suspect lesions are visualized due to the fact that certain tissues emit light with longer wavelengths after excitation by a short wavelength light source. In the prototype of AFI the mucosa was illuminated sequentially with the blue, green and red spectra of the light causing a green reflectance image without using fluorescent dyes [6]. More recently a trimodal imaging device combining Narrow Band Imaging (NBI), high definition WLE and AFI in a single endoscope has also been introduced to the market [7].

Another macroscopic technique which can be used for the detection of premalignant conditions is near infrared (NIR) imaging which uses an optimal near infrared light spectrum (i.e. from 650 nm to 900 nm). It has already been shown in a mouse model of colonic adenomatosis that a multichannel miniaturized NIR-endoscope can be useful for *in vivo* imaging of diverse molecular targets [8]. This might be a potential device for the future, however as of today there is no routinely available NIR endoscope applicable in humans.

For microscopic characterization confocal laser endomicroscopy (CLE) has been introduced to the market more than a decade ago [9]. As the technical principle, CLE is based on tissue illumination with a low-power laser with subsequent detection of light reflected through a pinhole, thereby leading to microscopic imaging with approximately 1000-fold magnification of the gastrointestinal mucosa *in vivo* [10].

In the past, two FDA-approved and CE-certified CLE systems have been available [11]: (i) a probe based CLE system (pCLE, Cellvizio, Mauna Kea Technologies, Paris, France) with different probes available for virtually any mucosal surface within the body which can be inserted into the working channel of a standard endoscope and (ii) an endoscope based system in which a confocal laser endomicroscope is integrated into the distal end of a high-resolution endoscope (eCLE, Pentax, Tokyo, Japan). Although the eCLE system has frequent distribution and is still used in clinical applications to date, it is no longer available. The pCLE system is based on stand-alone confocal probes and utilizes a fixed laser power and a fixed imaging plane depth for image acquisition. Depending of the confocal probe used, the lateral resolution of pCLE ranges between 3.5 μM and 1 μM with an image acquisition of 12 images/s thereby enabling real-time video microscopy of the intestinal mucosa.

In order to generate tissue fluorescence, intravenous and/or topically applied contrast agents are required for CLE imaging. Upon intravenous administration of fluorescein as the most commonly used contrast agent, blood vessels, the lamina propria, and intracellular spaces within the mucus are contrasted whereas cell nuclei are not stained with fluorescein. Nuclear staining usually requires administration of topical contrast agents such as acriflavine and cresyl violet [12,13]; however, there is concern over mutagenic potential with the topical agents [14].

2.2. Molecular probes

Apart from the endoscopic imaging device, fluorescent labeled or autofluorescent molecular probes are the second key component for molecular imaging. So far, different classes of molecular probes have been utilized for molecular imaging such as antibodies, peptides, aptamers, affibodies, nanoparticles or activatable probes [15]. Theoretically, the ideal molecular probe should exhibit fast binding kinetics with high specificity towards a defined molecular target and a high tissue penetration along with a short half-life in order to minimize systemic exposure [16,17].

Antibodies are among the most commonly used probes for molecular imaging with the advantage of highly specific binding. Further, with advances in antibody engineering and amino acid modification in the Fc fragment, serum half-life and specificity can be modified. On the other hand, antibodies bear the risk of immunogenicity and allergenic properties and, due to their large molecular weight of approximately 150 kDa, have a relatively poor tissue penetration. With mutations of their Fc domain, one can either increase or decrease the serum half-life without losing the ability of high binding affinity [18].

Peptides are short chained amino acids, which in contrast to the other molecular probes, are characterized by a low molecular weight and fast elimination [19]. Their advantage is that they are generally nontoxic, have minimal immunogenicity and a high affinity to their binding site [20]. Their short serum half-lives, usually caused by degradation or excretion together with their low toxicity makes them also topically applicable. On the other, the development of specific peptides is rather complex requiring high throughput methods or phage display while at the same time the exact binding site remains frequently unknown [21,22].

Aptamers are short single-stranded, oligonucleotides (RNA or ssDNA) that are capable of binding various molecules with high affinity and specificity [23,24]. In fact, aptamers are non-toxic nucleotide analogues of antibodies with the advantage that genera-

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