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TOPIC HIGHLIGHT

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Optical molecular imaging for detection of Barrett's-associated neoplasia

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Abstract

Recent advancements in the endoscopic imaging of Barrett's esophagus can be used to probe a wide range of optical properties that are altered with neoplastic progression. This review summarizes relevant changes in optical properties as well as imaging approaches that measures those changes. Wide-field imaging approaches include narrow-band imaging that measures changes in light scattering and absorption, and autofluorescence imaging that measure changes in endogenous fluorophores. High-resolution imaging approaches include optical coherence tomography, endocytoscopy, confocal microendoscopy, and high-resolution microendoscopy. These technologies, some coupled with an appropriate contrast agent, can measure differences in glandular morphology, nuclear morphology, or vascular alterations associated with neoplasia. Advances in targeted contrast agents are further discussed. Studies that have explored these technologies are highlighted; as are the advantages and limitations of each.

BACKGROUND

The incidence of esophageal adenocarcinoma (EAC) is rapidly increasing; over the last 40 years, the incidence rate of EAC has risen by over 300% in the United States[1]. This is of particular concern because the overall 5-year survival rate for patients diagnosed with EAC is only 12%[2]. Although detecting and treating esophageal neoplasia at an early stage has been reported to increase 5-year survival to rates as high as 81%[3], current methods of early detection have significant limitations. As a result, more than 60% of patients with EAC are diagnosed at a late stage, after local, regional, or distant metastases have occurred[4].

EAC arises primarily in patients with Barrett's esophagus (BE)[5,6], which is a highly prevalent condition in which the squamous epithelium of the esophagus is replaced by intestinal metaplasia (IM) near the gastroesophageal (GE) junction[7-9]. Because of this increased risk, patients with BE undergo regular surveillance endoscopy at designated intervals in an attempt to identify neoplastic lesions at an early stage[10,11]. Surveillance involves endoscopic examination with random four-quadrant biopsies

taken every 1-2 cm along the BE segment[10].

Despite surveillance efforts, routine biopsy protocols have been shown to miss up to 57% of neoplastic lesions in patients with BE[12]. This is largely due to the fact that dysplasia or neoplasia may be focal, flat and endoscopically indistinguishable from non-neoplastic epithelium on routine white-light endoscopy (WLE). The ability to delineate better superficial mucosal changes associated with early neoplasia at a macroscopic level, and subsequently, identify the subcellular changes associated with neoplastic progression would greatly enhance the yield and efficacy of current surveillance practices.

CHANGES IN OPTICAL PROPERTIES

In a standard WLE examination, the endoscopist views white light reflected from the surface of the esophagus; although visual examination of reflected white light can identify some changes in tissue morphology associated with neoplasia, it does not exploit the full range of changes in tissue optical properties that are associated with dysplasia and cancer. Neoplasia alters the light absorption and scattering properties of esophageal tissue[13,14]; in addition, neoplasia is associated with changes in the autofluorescence properties of esophageal tissue[13,15-17].

A number of new endoscopic approaches have been developed to more effectively probe neoplasia-related changes in optical properties to improve visualization of early neoplastic lesions. For example, the color of illumination light can be optimized to probe better changes in tissue absorption and/or scattering. Autofluorescence endoscopy can be used to image changes in tissue fluorescence that are associated with neoplasia. Moreover, improving spatial resolution of endoscopic imaging can help reveal changes in cellular architecture and morphology associated with neoplasia. Finally, optically active contrast agents can be used to improve further image contrast and probe specific molecular and morphologic features of neoplastic tissue that may not be associated with changes in native optical properties.

Here, we first review changes in the optical properties of esophageal tissue associated with neoplasia, and then outline new endoscopic imaging approaches to use these changes to improve early detection of esophageal neoplasia. Finally, we discuss the use of targeted contrast agents to expand the range of molecular changes that can be imaged *in vivo*.

Neoplasia-associated changes in tissue light scattering and absorption

Light attenuation in esophageal tissue is governed by a combination of absorption and scattering. In the visible region of the spectrum, the primary source of light absorption in esophageal tissue is hemoglobin. Esophageal neoplasia is associated with increased angiogenesis[18], and endoscopic imaging approaches to enhance vascular contrast may improve early detection[19,20]. Oxyhemoglobin has absorption peaks at 420, 542, and 577 nm[13]; examining the tissue at these illumination wavelengths can enhance vascular contrast, with vasculature appearing visibly darker than the surrounding tissue due to the increase in light absorption[21]. Neovascularization is an important quantifiable tool for distinguishing neoplasia from non-neoplastic Barrett's epithelium. Irregular angiogenesis occurs within the lamina propria at various levels of the mucosal layer in high-grade dysplasia (HGD) and cancer. These features have been verified by analysis of microvessels and overexpression of relevant markers such as vascular endothelial growth factor and CD34, which results in a statistically significant difference between the microvessel density in BE versus HGD and cancer[19,20].

Light scattering in tissue is a result of spatial fluctuations in the refractive index. In general, the scattering of stroma is significantly greater than that of the epithelium and is the dominant source of reflected white light from intact tissue. Neoplasia is associated with a small decrease in stromal scattering that is attributed to degradation in collagen fibers, possibly due to proteases secreted by pre-neoplastic epithelial cells[18,22,23]. The attenuation of light in tissue is wavelength dependent, with longer red wavelengths able to penetrate more deeply than shorter blue wavelengths. Thus, tuning the illumination wavelength provides some ability to control penetration depth, and highlight vascular contrast.

Neoplasia-associated changes in tissue autofluorescence

Some endogenous constituents of esophageal tissue can remit absorbed light in the form of fluorescence. Endogenous fluorophores are found in both the epithelium and the stroma of esophageal tissue, and fluorescence imaging provides a way to monitor changes in the concentration and composition of these fluorophores. When esophageal tissue undergoes malignant transformation, endogenous fluorophores undergo alterations[24-26], which can be probed *via* autofluorescence imaging (AFI), to detect abnormalities that may not be visible during standard WLE. Tuning the excitation wavelength provides a way to selectively probe various fluorophores that can then be quantified by measuring light intensity at specific emission wavelengths[26].

The primary fluorophores within the epithelium include mitochondrial NADH and FAD found in epithelial cells. Epithelial cells show cytoplasmic autofluorescence attributed to NADH using UV excitation wavelengths (330-370 nm) and FAD using green excitation wavelengths (510-550 nm)[27,28]. Levels of mitochondrial NADH[15] and mitochondrial FAD increase due to dysplastic changes in the epithelium[29,30].

Stromal fluorescence of esophageal tissue is predominantly associated with covalent collagen crosslinks, which are characterized by relatively high autofluorescence intensity across a broad range of UV, blue, and green excitation wavelengths[16]. Esophageal neoplasia is associated with a loss of stromal autofluorescence, which has been attributed to a decrease in collagen crosslinking[18,22,23]. Finally, invasive esophageal cancers are often associated with porphyrin fluorescence, with maximal excitation near 400 nm and emission in the red spectral region[13,31,32].

Table 1 Advantages and disadvantages of optical technologies for identification of neoplasia in Barrett's esophagus

Technology	Advantages	Disadvantages	Stage of clinical translation
Standard WLE	Capable of scanning wide area, widely available outside of tertiary care centers, no exogenous contrast	Limited sensitivity and specificity	Commercially available
High-definition WLE	Capable of scanning wide area, increased image contrast, no exogenous contrast	Performance evaluated in moderate-sized studies	Commercially available
AFI	Capable of scanning wide area, consistently high sensitivity, no exogenous contrast	High rate of false positives, performance evaluated only in small pilot studies	Commercially available
NBI	Capable of scanning wide area, consistently high sensitivity, no exogenous contrast	Performance evaluated in small pilot studies	Commercially available
OCT	Resolves subsurface structure, no exogenous contrast	Technology still under development	Clinical studies
Endocytoscopy	Histology-like imaging, high specificity	Low sensitivity, limited field of view, requires exogenous contrast	Commercially available
CME	Nuclear morphology can be viewed, high sensitivity and specificity	Limited field of view, high cost, uses IV exogenous contrast	Commercially available
High-resolution microendoscopy	Some nuclear morphology can be viewed, lower cost, adaptable to any endoscope	Limited field of view, requires exogenous contrast, technology still in development	Clinical studies

AFI: Autofluorescence imaging; OCT: Optical coherence tomography; CME: Confocal microendoscopy; WLE: White-light endoscopy; NBI: Narrow-band imaging.

High-resolution imaging

The spatial resolution of optical imaging is governed by diffraction, and with visible wavelengths of light, subcellular resolution imaging is possible. Typically, standard endoscopic imaging approaches do not achieve diffraction-limited resolution, however, recent advances in high-resolution imaging techniques such as optical coherence tomography (OCT), endocytoscopy, and endomicroscopy afford the ability to image with subcellular resolution. Such approaches are often termed "optical biopsy", because they allow visualization of glandular and cellular alterations associated with neoplasia. Optical contrast in high-resolution imaging is governed by the same alterations in tissue absorption, scattering and fluorescence described above. In addition, optically active contrast agents are often used to increase contrast for high-resolution imaging.

Table 2 Summary of performance of emerging optical technologies

Type of detection	Study size	Sensitivity, specificity
AFI	60 patients, 116 images	91%, 43%[36]
NBI	63 patients, 175 images	94%, 76%[33]
	51 patients, 204 images	100%, 98%[34]
	21 patients, 75 images	89%, 95%[38]
High-resolution imaging (1-15 μm resolution)		
OCT	33 patients, 314 images	68%, 82%[43]
	55 patients, 177 images	83%, 75%[58]
Endocytoscopy	16 patients, 166 images	56%, 68% (425 ×) 42%, 83% (1125 ×)[47]
Confocal imaging	63 patients, 433 images	93%, 98%[48]
	38 patients, 296 images	75%, 90%[50]

AFI: Autofluorescence imaging; OCT: Optical coherence tomography; NBI: Narrow-band imaging.

IN VIVO ASSESSMENT OF IMAGING TECHNOLOGIES

In the past decade, advances in imaging technologies have enabled gastroenterologists to optically image Barrett's-associated neoplasia with better contrast *in vivo*. The development of wide-field imaging technologies affords clinicians a macroscopic view of the tissue, serving as a "red-flag technique" for relevant abnormalities. High-resolution technologies assess microscopic features of the tissue and, if coupled with an ideal source of contrast, may measure biochemical, molecular, and vascular changes. Table 1 summarizes a number of different optical technologies currently under investigation, describes the advantages and disadvantages of each, and describes which stage they have reached in terms of clinical translation. Table 2 summarizes the accuracy of the technologies that have been translated to clinical use and have been used in large clinical trials.

Narrow-band imaging

Narrow-band imaging (NBI) is a wide-field imaging technology that takes advantage of changes in light scattering and absorption in neoplastic tissue. Systems that implement NBI illuminate tissue with one or more narrow-band wavelength ranges corresponding to hemoglobin absorption peaks. Reflected light in these bandwidths is recombined to create a digital image with enhanced vascular contrast. This approach can also enhance visualization of villous mucosal patterns due to lining of vessels in mucosal folds[21]. An example is shown in Figure 1.

For example, one NBI system combines information from three wavelength ranges: 400-430 nm (blue), 530-550 nm (green), and 600-620 nm (red). Higher relative intensity from the blue region is used to enhance surface level vasculature associated with neoplasia, due to its shallow penetration depth. In a 63 patient study using this approach, researchers in Amsterdam used features such as mucosal morphology and vascular contrast to determine grade of disease. The presence and regularity of these patterns were found to be essential for image evaluation. Out of the 175 areas, 52 were used as training material for endoscopists and the remaining 123 were used as a validation set. In the validation set, 94% of HGD images were

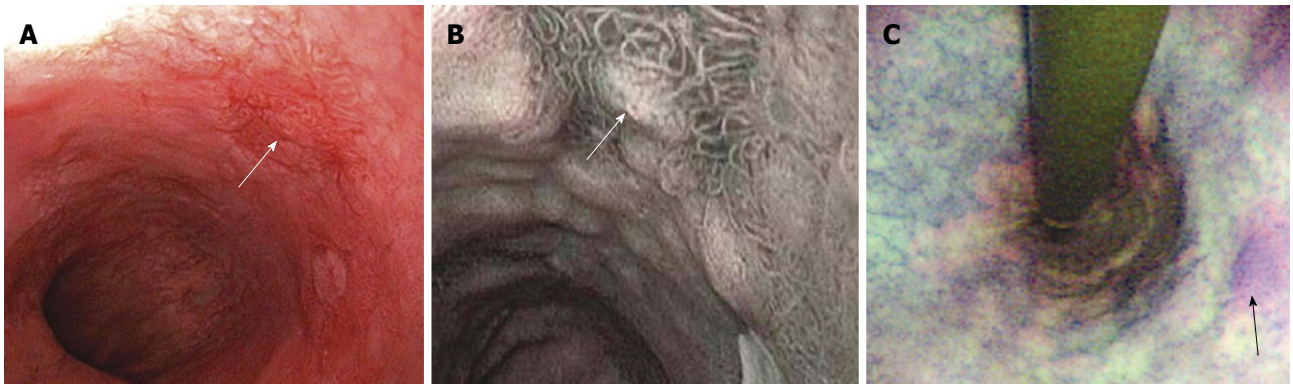

Figure 1 Endoscopic images from an area positive for esophageal adenocarcinoma. Abnormal areas (arrow) can be seen in the high-resolution white light image (A), and the narrow-band image (B) [Copyright (2008), with permission from Elsevier][38]; in the narrow-band image, the irregular mucosal morphology is visible (arrow); an abnormal area (arrow) can be seen in the autofluorescence image (C) where areas with loss of fluorescence are indicated as purple regions in the pseudo-colored overlay [Copyright (2005), with permission from Elsevier][36].

noted to show irregular or disrupted villous/gyrus mucosal pattern, and 85% were noted to show irregular vascular patterns. Using these features and others, they developed a multi-step hierarchical classification system based on mucosal morphology, including features such as type and regularity of mucosal patterns, regularity of vasculature patterns, and presence and type of abnormal blood vessels. Using this multistep evaluation, they determined the overall sensitivity and specificity to be 94% and 76%, respectively[33]. Similarly promising performance was also obtained using the same NBI system in a 51 patient study by Sharma and colleagues; sensitivity and specificity for detection of HGD were 100% and 99%, respectively[34].

Of continued debate, however, is the question of how NBI compares to high-definition white-light endoscopy (HD-WLE) using the current generation of endoscopes. This new generation of endoscopes offers markedly higher pixel densities and high-definition images that result in increased contrast in villous mucosal patterns, and a marked improvement in resolution[35,36] over standard WLE[37]. In a study with 65 patients, Wolfsen and colleagues, using a narrow-band system in which only two of the shorter wavelength ranges associated with hemoglobin were used, observed that the combination of HD-WLE and NBI did find higher grades of dysplasia in 18% of the study patients, using fewer biopsies than for standard endoscopy. They also observed that out of five of the cases in which HGD or EAC was detected, three were detected by HD-WLE as well. Although results favored NBI, the study was not designed to determine the efficacy of one modality over the other[38]. Another study by Curvers and colleagues has observed that, while expert endoscopists preferred the image contrast provided by NBI, this did not improve overall interobserver agreement or accuracy when compared to HD-WLE[39,40]. Larger studies are needed to determine which is the more accurate technique.

AFI

AFI can also increase contrast between non-neoplastic and neoplastic sites, as a result of the loss of autofluorescence associated with esophageal neoplasia. Typically, tissue autofluorescence is excited in the blue region (395-475 nm) and fluorescence emission is collected at longer wavelengths (> 490 nm) to detect changes in fluorophores associated with malignant transformation. Because the intensity of autofluorescence can be low, this technique requires the use of highly sensitive CCDs to collect the autofluorescence signal. In recent systems, reflected light is also collected through a second CCD. Co-registered images can be used to compensate for changes in fluorescence intensity associated with variations in illumination and distance from the tip of the endoscope to the tissue, thereby further enhancing autofluorescence contrast. The resulting effect is pseudo-colored purple to highlight neoplastic lesions[36,37]. An example is shown in Figure 1.

In a recent 60 patient study using a standard endoscope with an added AFI component, Kara was able to detect HGD in 22 patients, 14 of which were detected with AFI and WLE, and six of which were detected using AFI alone; thereby increasing the detection rate from 23% to 33% using AFI. Only one of the patients was diagnosed using four-quadrant biopsies alone[36]. Results suggest that AFI may aid in the detection of additional HGD sites; however, it may not exclude the need for the standard four-quadrant biopsies. Sensitivity and specificity based on the 116 samples used for this study were 91% and 43%, respectively. Although no patient was diagnosed without AFI and four-quadrant biopsies, they cite a high rate of false positives using AFI alone, due in part to the loss of autofluorescence associated with acute inflammation[36].

Although individually these enhanced endoscopic technologies have shown success, the high rate of false positives is a major drawback. To address this limitation, a combination of modalities is being explored to utilize the benefits of each; potentially increasing the accuracy of detection at the point of surveillance. Kara and colleagues have conducted a 20 patient pilot study in which HD-WLE and AFI were used initially to locate suspicious lesions. Once the lesions were identified, an NBI scope was introduced for detailed inspection of vascular and mucosal patterns. They found that 40% of the HGD lesions were discovered with AFI alone. However, the false-positive rate of

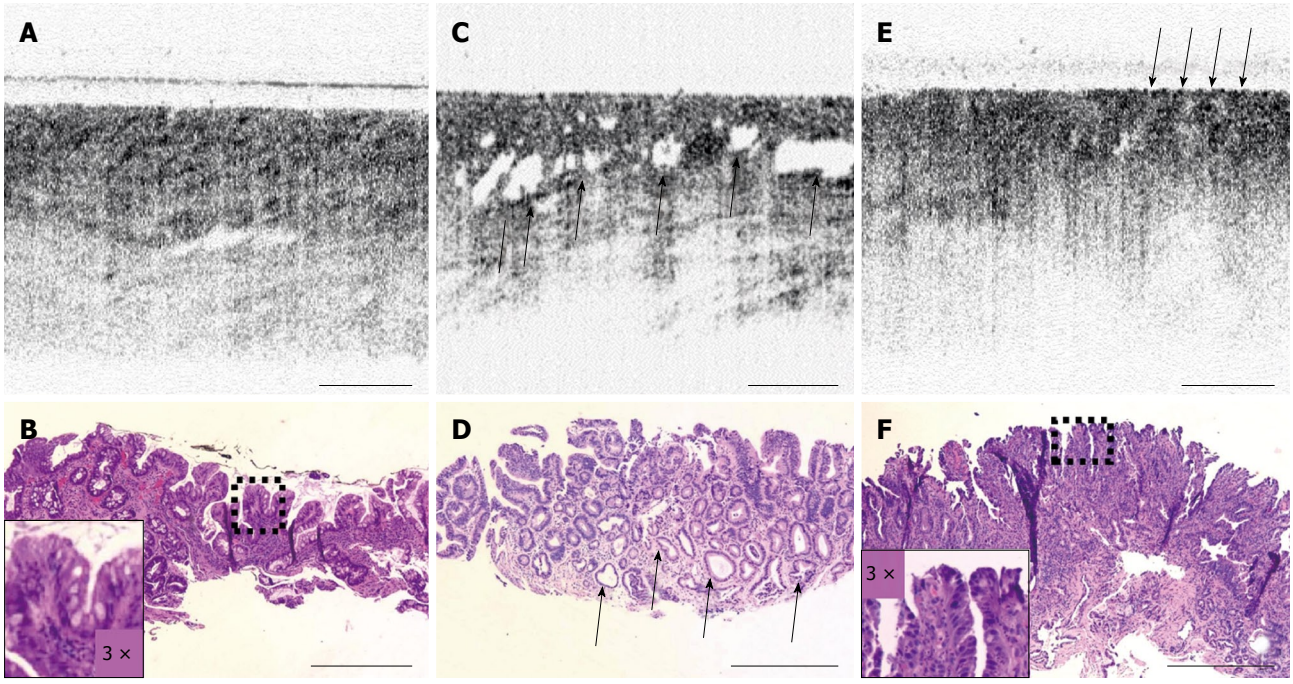

Figure 2 Optical coherence tomography images of intestinal metaplasia (A), and of neoplasia (C, E) are shown with corresponding histological images shown below [Copyright (2006), with permission from Elsevier][43], dilated glands (C) and increased surface reflectivity (E) can be seen in the optical coherence tomography images of neoplastic tissue, corresponding histopathology is shown (B, D, F). Scale bars, 500 μm.

the modality was 40% and the positive predictive value was 60%. Following NBI inspection, the false-positive rate was reduced to 10%, which achieved a positive predictive value of 85%[41]. A more recent study with one scope containing both modalities achieved similar results. In that study, AFI was able to detect more lesions than high-resolution WLE alone, however, the false-positive rate remained a high 81%; following detailed inspective with NBI the rate was reduced to 26%[42]. In both cases however, random four-quadrant biopsies detected additional lesions that the optical modalities did not identify, which indicates the need for further development of these and other technologies.

High-resolution imaging

Wide-field imaging techniques, such as AFI and NBI, were developed to measure large surface areas of gastrointestinal tissue. More recently, high-resolution systems have been developed to achieve near diffraction-limited imaging from small fields of view. Four primary approaches have been pursued to increase spatial resolution. OCT can image esophageal tissue with 10-15 μm resolution and a penetration depth of 1-2 mm. Endocytoscopy can image surface level esophageal tissue with up to 1-2 μm resolution using the highest magnification setting. Confocal microscopy can image esophageal tissue with 1-2 μm spatial resolution with a penetration depth of 300-400 μm. High-resolution microendoscopy can image surface level esophageal tissue with 4-5 μm spatial resolution. Recent clinical studies with these modalities highlight the benefits and limitations of high-resolution imaging.

OCT uses variations in the time it takes light to be reflected from structures beneath the tissue surface to image sub-surface tissue structures as seen in Figure 2, in a manner analogous to ultrasound imaging. In a 55 patient study, researchers have determined that OCT could differentiate HGD and EAC from BE with a sensitivity of 83% and a specificity of 75%[43]. An advantage of OCT is that it relies on endogenous differences in light scattering to generate image contrast. OCT may be a particularly useful tool in the detection and surveillance of sub-squamous BE because of its relatively greater depth of penetration[44]. However, the technology is still under development[45] and further clinical studies are needed to assess performance in a wide variety of clinical settings.

Endocytoscopy uses a probe that is passed through the instrumentation channel of an endoscope to image with subcellular resolution. Essentially, high-resolution epi-reflectance microscopy is used with methylene blue contrast to highlight relevant nuclear features (Figure 3A and B). Although models vary, there are generally two types each with different magnifications settings; one at 450 × where the field of view can be as wide as 300 μm × 300 μm, and a higher magnification setting of 1125 × where a field of view as small as 120 μm × 120 μm is made visible[46]. A large study evaluating 166 sites in 16 patients with endocytoscopy by Pohl and colleagues reported a sensitivity and specificity of 42% and 83%, respectively[47]. Although high specificity was encouraging, they did emphasize the need for an initial wide-field surveillance technique to identify suspicious areas. This technology is certainly promising; however, larger studies need to be performed.

Confocal microendoscopy (CME) images subsurface tissue structure with high resolution by using a spatial filter to reduce the background signal produced by scat-

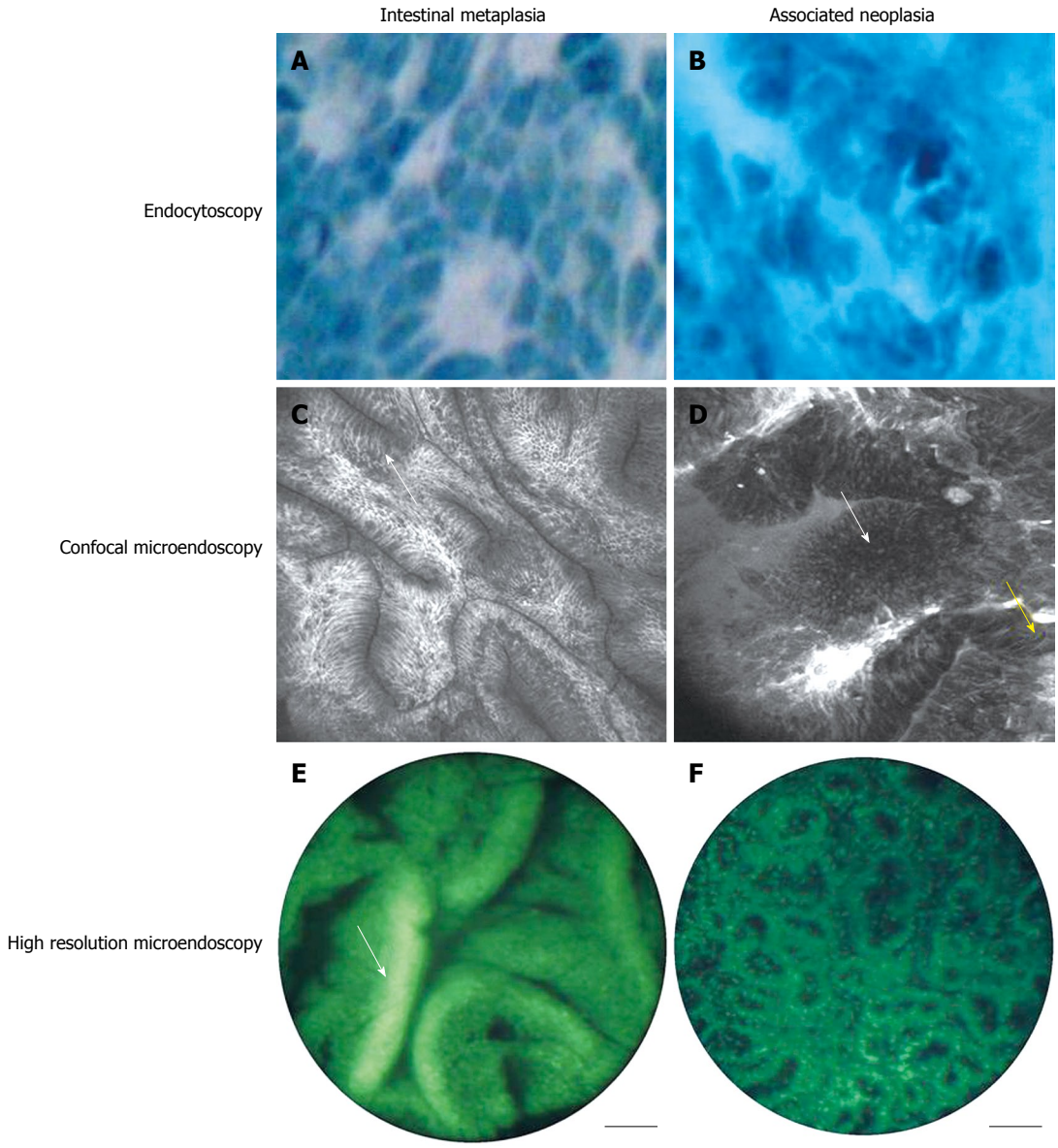

Figure 3 Images representing intestinal metaplasia and neoplasia collected using endocytoscopy (A, B) (Copyright (2007), with permission from Thieme)[47], confocal microendoscopy (C, D) [Copyright (2006), with permission from Elsevier][48], and high-resolution microendoscopy (E, F) [Copyright (2008), with permission from Elsevier][51]. Topically applied methylene blue is used in endocytoscopy to highlight nuclear changes (A, B); In metaplasia (A), nuclei appear organized and regular; this is in stark contrast to neoplasia (B) where nuclei appear pleomorphic. Both images were taken using 1125 × magnification. Confocal images were taken using intravenous fluorescein to enhance contrast of subepithelial capillaries (C, D); for intestinal metaplasia (C), confocal microendoscopy allows visualization of mucin-containing goblet cells (white arrow); For Barrett's-associated neoplasia (B), cells are irregularly oriented (white arrow) and malignant invasion of the lamina propria can be seen (yellow arrow). Confocal images are 500 μm × 500 μm. High-resolution microendoscopy uses proflavine for contrast enhancement, highlighting changes in glandular and nuclear patterns (E, F). High-resolution images are 750 μm in diameter.

tered out-of-focus light, which produces images with 1-2 μm spatial resolution. Although CME images can be generated either in reflectance or fluorescence mode, in the context of esophageal imaging, fluorescence CME has been primarily used. Since tissue autofluorescence is weak, typically fluorescent contrast agents are used to generate image contrast in CME. Kiesslich and researchers conducted a 63 patient study in Germany using an endoscope that incorporated standard WLE and confocal microscopy; fluorescein (10% w/v) was administered intravenously to generate vascular contrast. Subepithelial capillaries located in the upper and deeper layers of the lamina propria were identified due to fluorescein contrast. Leakage of fluorescein due to irregular capillary formation indicated neoplastic areas (Figure 3C and D). Indeed, due to these irregularities, neoplasia could be detected with a sensitivity and specificity of 94% and 98% respectively[48]. In a prospective, randomized, double-blind, controlled, crossover study with 39 patients using the same system, CME with targeted biopsy was shown to not only be accurate, but to nearly double the diagnostic yield of collected biopsies. In examining the biopsies identified by standard four-quadrant biopsies and the biopsies identified by CME, there was no statistically significant difference in detection of neoplasia between the two techniques[49]. However, although accuracy and

diagnostic yield is impressive, the high cost may limit this technology to tertiary care centers.

A fiber-bundle, probe-based confocal system that can be passed through the instrument channel of any standard endoscope was used in a 38 patient study by Pohl and other researchers. A major benefit of this technology is its adaptability to existing endoscopes. This system also requires exogenous contrast; fluorescein was administered intravenously. The sensitivity and specificity of the two study endoscopists were 75% and 89% and 75% and 91%, respectively. They concluded that the confocal fiber probe showed a high negative predictive value for detecting unapparent neoplasia in BE; however, sensitivity was not ideal[50].

An alternative approach to high-resolution fluorescence imaging uses a coherent fiber bundle placed in direct contrast with the surface of tissue labeled with fluorescent dyes to yield high resolution images that reveal subcellular structure (Figure 3E and F)[51]. This low-cost alternative to confocal imaging may be suited for community-wide surveillance outside of tertiary care centers. In a small pilot study of nine patients, with topical proflavine for contrast enhancement of cell nuclei, researchers achieved a sensitivity and specificity of 87% and 85% using fluorescence microendoscopy[52].

Contrast enhancement

As optical imaging technology continues to advance, the concurrent development of appropriate contrast agents that target biomarkers of neoplasia is crucial. Two general classes of optical contrast agents have been explored to improve image contrast: vital dyes and targeted contrast agents. Absorbing or fluorescent dyes that have an affinity for specific tissue constituents have often been used to improve the ability to visualize specific features associated with neoplasia. Often referred to as vital dyes, these stains can help delineate features such as angiogenesis, leaky vasculature, and cell morphology. In contrast, targeted contrast agents use a high affinity probe molecule to target a specific molecular biomarker associated with neoplasia[53]. The probe molecule must be coupled to an optically active component, such as a fluorescent dye or scattering nanoparticle. Here, we briefly review the utility of both types of contrast agents for improved detection of esophageal neoplasia.

Vital dyes can be utilized to delineate better morphological changes associated with epithelial neoplasia. For example, the absorptive dye methylene blue localizes primarily in nuclei and can enhance visualization of nuclei when coupled with appropriate high-resolution instrumentation. Using an endocytoscope, nuclear characteristics associated with neoplasia such as homogeneity, nuclear-to-cytoplasmic ratio, and organization can be resolved. However, since methylene blue dye is known to induce oxidative damage of DNA when exposed to white light illumination[54], the risks of the contrast agent need to be weighed against the benefits to determine potential use.

Fluorescent vital dyes may be advantageous due to the lack of interference with standard endoscopy. Fluorescein is a dye that is administered intravenously, thus enhancing the view of vasculature in epithelial tissue. When coupled with confocal imaging, subsurface vasculature can be seen. The illumination and collection wavelengths of commercially available confocal systems correspond to fluorescein excitation (about 490 nm) and emission (about 520 nm)[48,50]. Acriflavine is another vital fluorescent dye that can be seen using similar excitation (about 450 nm) and emission (about 510 nm) wavelengths. Acriflavine stains cell nuclei, highlighting nuclear characteristics such as size, shape, and spacing, and has been used previously *in vivo* for gastrointestinal imaging[55].

Targeted contrast agents serve as beacons that signal specific molecular events associated with pre-cancer formation. The benefit of targeted agents is the potential to achieve a high signal to background ratio by virtue of selective binding to a molecular target. Lu and researchers used a phage display library with about 2.8×10^9 unique sequences to select a cancer-specific peptide. The library was biopanned against three cultured human esophageal cell types: adenocarcinoma, metaplasia, and normal, to identify a peptide with specificity for the adenocarcinoma cell line. They used the selected peptide labeled with FITC to image Barrett's-associated neoplasia *in vivo*. The agent was topically applied and imaged with a concurrently developed prototype fluorescence endoscope. Initial results showed a significant increase in binding to Barrett's-associated neoplasia over Barrett's alone when imaged with wide-field fluorescence imaging (Figure 4)[56]. In a different study, Hsiung and colleagues fluorescently labeled a high-affinity heptapeptide sequence selected with similar phage display techniques for the colon, and were able to differentiate dysplastic from non-dysplastic colonic crypts using confocal imaging[57]. In both of these cases, the topically applied contrast agent was incubated *in vivo* for a short period of time before the unbound agent was washed off to reduce non-specific signals. Although the excitation and emission wavelengths of these agents correspond well with commercially available confocal endoscopes, another important advantage demonstrated by these studies is the ability to image these agents with both wide-field fluorescence and CME.

DISCUSSION

Recent advances in imaging technologies afford visualization of endogenous optical alterations associated with gastrointestinal neoplasia. NBI shows contrast associated with light absorption due to hemoglobin. High sensitivity and specificity is cited in studies using this technology, however some indicate that there is no significant difference between contrast associated with NBI imaging and HD-WLE, which is becoming increasingly available. AFI measures the signal decrease associated with loss of stromal collagen fluorescence and increased fluorescence associated with porphyrin. Various studies evaluating AFI have cited high sensitivity, but a high rate of false positives. The combination of NBI and AFI may afford better sensitivity and specificity rates; NBI has shown to reduce

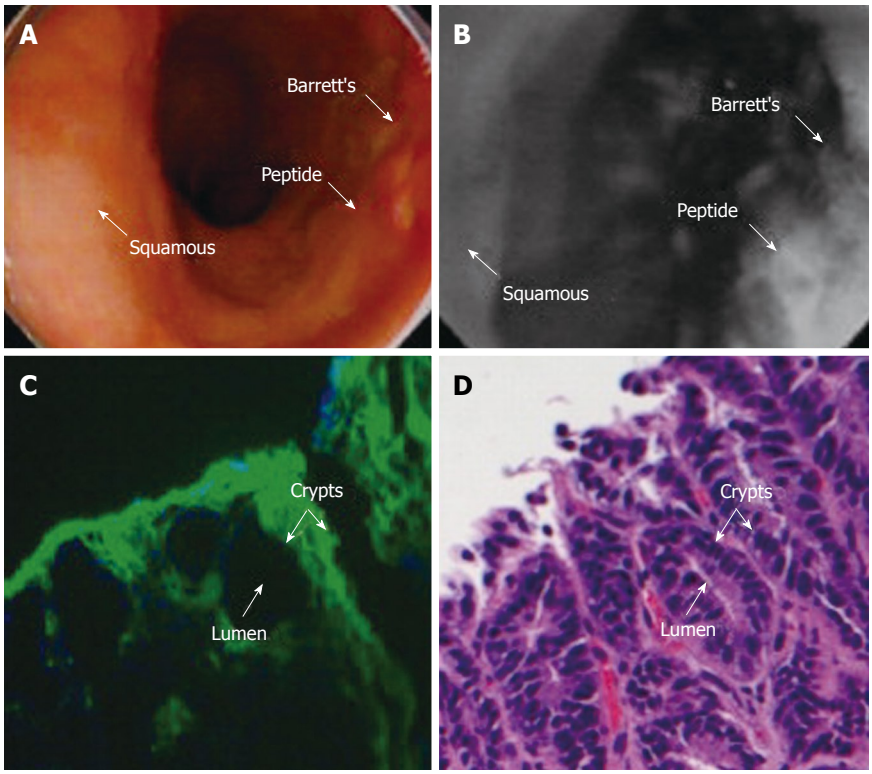

Figure 4 ***In vivo*** **localization of contrast agent localized to a neoplasia region visualized using wide-field fluorescence endoscopy.** White light endoscopic image (A) shows no evidence of lesion; topical administration of peptide-targeted fluorescent dye reveals neoplastic area (B) [Copyright (2008), with permission from IOS Press][56]; targeted neoplastic crypts seen with fluorescence microscopy (C), and corresponding histology (D) [Copyright (2010), with permission from Elsevier][59].

the number of false positives identified by AFI from 81% to 26%[42].

High-resolution imaging will also play a major role in improving detection, affording clinicians an "optical biopsy" of epithelial tissue. Confocal imaging allows for optical sectioning of up to 250 μm deep, and coupled with vital dyes such as fluorescein, allows evaluation of vascular regularity. High sensitivity and specificity have been cited; however, high cost and the limited field of view remain concerns. Endocytoscopy allows for histology-like reflectance imaging where nuclei appear dark blue due to methylene blue contrast. The technology achieves high specificity; however, the dye has been shown to interfere with white light imaging and image quality has been an issue. When combined with wide-field imaging techniques, high-resolution technologies may reduce false-positive rates if coupled with the appropriate contrast agent.

Unfortunately, despite all the advances in optical imaging methods, there are still lesions that are only detected by standard four-quadrant biopsies. Improvements in contrast agents are also needed to facilitate early detection. A number of contrast agents are commercially available; primarily vital dyes such as fluorescein and methylene blue. However, recent *in vivo* testing of optically labeled high-affinity peptide and heptapeptide sequences has paved the way for molecule-specific contrast agents for gastrointestinal neoplasia[56,57]. Although advances have translated the use of vital dyes and contrast agents *in vivo*, there are still many unanswered questions regarding their ultimate clinical role. What will be the ideal mechanism of delivery? How will the development of *in vivo* imaging technologies accommodate the use of new contrast agents? Finally, will the addition of contrast agents create a multifaceted platform that can improve overall accuracy of surveillance?

Although these new imaging technologies may be appropriate for tertiary care centers, additional considerations are necessary as these technologies are disseminated more widely. A potential solution may be a lower cost technology such as the high-resolution microscope, or an adaptable technology such as the confocal miniprobe with topically applied contrast agents; both of which have been cited to achieve reasonably high sensitivity and specificity. Objective, quantitative algorithms will also be important because clinicians outside of tertiary care clinics may not be as familiar with optical characteristics of abnormal lesions detected with new technologies. Various groups have begun work in this area; however, larger trials need to be conducted to determine effectiveness[52,58].

At this point, larger studies are needed to test the combination of multi-scale, multi-modal technologies against the current surveillance standard, and to test whether the use of contrast agent is advantageous. This multifaceted optical approach has the potential to improve surveillance in BE; once validated, it has the potential to be utilized for surveillance of neoplasia along the gastrointestinal tract and can be further developed for screening.

DISCLOSURE STATEMENT

Thekkek N and Anandasabapathy S have no financial

conflicts to disclose. One of the co-authors (Richards-Kortum R) has a small ownership interest in Remicalm, Inc which has licensed related technology from the University of Texas at Austin and Rice University.

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