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TOPIC HIGHLIGHT

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Molecular markers and imaging tools to identify malignant potential in Barrett's esophagus

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Abstract

Due to its rapidly rising incidence and high mortality, esophageal adenocarcinoma is a major public health concern, particularly in Western countries. The steps involved in the progression from its predisposing condition, gastroesophageal reflux disease, to its premalignant disorder, Barrett's esophagus, and to cancer, are incompletely understood. Current screening and surveillance methods are limited by the lack of population-wide utility, incomplete sampling of standard biopsies, and subjectivity of evaluation. Advances in endoscopic ablation have raised the hope of effective therapy for eradication of high-risk Barrett's lesions, but improvements are needed in determining when to apply this treatment and how to follow patients clinically. Researchers have evaluated numerous potential molecular biomarkers with the goal of detecting dysplasia, with varying degrees of success. The combination of biomarker panels with epidemiologic risk factors to yield clinical risk scoring systems is promising. New approaches to sample tissue may also be combined with these biomarkers for less invasive screening and surveillance. The development of novel endoscopic imaging tools in recent years has the potential to markedly improve detection of small foci of dysplasia *in vivo*. Current and future efforts will aim to determine the combination of markers and imaging modalities that will most effectively improve the rate of early detection of high-risk lesions in Barrett's esophagus.

Key words: Barrett's esophagus; Esophageal adenocarcinoma; Gastroesophageal reflux disease; Dysplasia; Biomarkers; Endoscopic imaging

Core tip: This review highlights recent advances and future directions in biomarker development and endoscopic imaging technology for identification of patients at risk of malignant progression of Barrett's esophagus.

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INTRODUCTION

Esophageal adenocarcinoma (EAC) has increased in incidence in the United States and other Western countries by at least six-fold in the past three decades, making it the cancer with the most rapid rise in incidence[1]. Prognosis is dismal at the time of diagnosis, with a five-year survival rate that remains below 20%[2]. This is particularly sobering in light of the longstanding recognition of Barrett's esophagus as a premalignant condition and of the technological advancements allowing for improved early detection and intervention.

Barrett's esophagus (BE) is defined as a replacement of normal squamous epithelium in the esophagus with columnar mucosa (endoscopic diagnosis), which is confirmed by biopsy as intestinal metaplasia (histologic diagnosis). Debate persists regarding the histologic requirement (such as presence of goblet cells) as well as the lack of distinction between short and long segment BE[3]. It is the leading risk factor for EAC, conferring a relative risk of 30-60 compared with that of the general population[4]. The pathophysiology of Barrett's metaplasia is incompletely understood but is related to chronic damage from gastric acid and bile reflux[5]. Strong association has been demonstrated between chronic gastroesophageal reflux disease (GERD) and both BE and EAC[6-8], but the nature of the progression from GERD to BE to EAC is less clear[9].

While BE is found in 5%-10% of patients with chronic GERD, most patients do not progress to EAC[10]. Moreover, most EAC are diagnosed incidentally, without a known history of GERD or BE[11], and quite often in advanced stages less amenable to cure. Thus from a public health standpoint, the key questions are: which members of the general population should be screened for BE, which patients with BE are likely to progress to EAC, and what surveillance program is appropriate[12]. In this review, we discuss the current understanding of Barrett's progression, recent advances in biomarker and endoscopic imaging development, and implications for future research and clinical practice.

EPIDEMIOLOGICAL MARKERS

In addition to chronic GERD, several risk factors for BE are well-established, including age over 50 years, male sex, white race, obesity, intra-abdominal fat distribution, and presence of hiatal hernia. Screening endoscopy may be appropriate for patients meeting several of these criteria[3,13]. Unfortunately, the vast majority of patients diagnosed with EAC have no prior diagnosis of BE, and many patients diagnosed with BE have no prior GERD symptoms[9].

CURRENT SURVEILLANCE PRACTICES

Current United States society guidelines recommend endoscopic surveillance of patients with documented BE[3,9,13] for the presence of EAC or its precursor lesions low-grade or high-grade dysplasia (LGD or HGD, respectively). This consists of regularly scheduled white light endoscopy with four-quadrant biopsies taken at 2 cm intervals, or 1 cm in patients with known or suspected dysplasia (Seattle protocol)[13]. Even when applied rigorously, this approach samples only a small fraction of the mucosal surface, and retrospective evidence suggests that in practice, the number of biopsies taken is often considerably lower than recommended, creating further sampling error[14]. This is especially problematic since early dysplastic lesions typically occur as small foci and can readily evade detection by the standard endoscopic biopsy practice regimen. Furthermore, these biopsy samples are typically not fully sectioned and examined; instead only a few sections from each sample are reviewed, which represents yet another order or two of magnitude decrease in actual tissue examined[15]. The present definitions of LGD and HGD are based on morphologic distinctions as graded by a pathologist; although interobserver reproducibility has been shown to be high at the ends of the spectrum (BE *vs* HGD or EAC), there appears to be considerable variation in separating nondysplastic BE from LGD or indeterminate dysplasia[12,16]. This non-concordance is even greater in the community setting, where a recent study demonstrated marked over-diagnosis of LGD following review of samples by a panel of expert pathologists[17].

These distinctions are important in practice because they have bearing on the likelihood of progression to EAC and consequently the need for close surveillance or intervention. For example, in the aforementioned study, patients with a consensus histologic diagnosis of LGD went on to develop HGD or EAC at a rate of 13% per year, whereas those downgraded to nondysplastic BE (NDBE) progressed at a rate of only 0.49% per year[17], although other studies suggest a lower incidence of LGD to HGD/EAC progression[18,19]. This is in keeping with data from recent large multicenter studies and meta-analyses, which estimate a low overall rate of progression from NDBE to EAC, on the order of 0.12%-0.38% per year, with very low mortality from EAC[19-21]. These findings, coupled with a lack of strong evidence showing mortality benefit, have led some health economists to argue that routine endoscopic surveillance of all patients with BE is likely not cost-effective[22], although at present it remains supported by guidelines[3,13].

ADVANCES IN THERAPY

Recent years have also seen the development and evaluation of endoscopic ablative techniques for dysplastic BE, which hold the promise of cancer prevention analogous to the current practice of polyp resection in the colon. Endoscopic mucosal resection has proven to be an effective therapeutic intervention in many patients with HGD or even intramucosal carcinoma and is associated with lower morbidity than surgical resection, although risk of cancer recurrence is higher in patients with lesions not strictly confined to the mucosa[23,24]. Radiofrequency ablation (RFA) has been shown to have high efficacy in the eradication of dysplasia and intestinal metaplasia as well as a good safety profile[25], and this effect appears to be durable[26]. In light of these encouraging findings and the high mortality of EAC, some experts have reintroduced the question of whether all BE should be ablated[27,28]. At present, while it appears to be cost-effective to ablate all HGD, it is less clear whether ablation of all LGD or NDBE is reasonable public health policy[29]. In addition, such efforts are complicated

by the presence of subsquamous intestinal metaplasia (SSIM), or "buried Barrett's," which can persist after ablation, is difficult to detect using current practice methods, and whose significance as a premalignant condition is as yet undetermined[30].

NEED FOR NEW BIOMARKERS

In this context, the main unresolved issue in BE management is to improve identification of those patients at highest risk for developing EAC.

The term "biomarker" broadly encompasses physiologic measurements, molecular analyses, or endoscopic or imaging findings[31]. The National Cancer Institute has established an Early Detection Research Network, which has developed a recommended biomarker validation pipeline encompassing a discovery phase, translational phase, and clinical implementation phase[32]. An ideal biomarker would objectively detect all dysplastic BE without significant false-positive results leading to unnecessary testing and intervention. As discoveries of such markers are few and far between, it is more realistic to expect that some combination of less-perfect markers will ultimately prove useful for the risk stratification of patients with BE. Many of the recent advances in biomarker research can be grouped into the categories of molecular markers and endoscopic imaging tools.

MOLECULAR MARKERS FOR DYSPLASTIC PROGRESSION

Much effort has been devoted in recent years to the search for a molecular marker that can serve as an adjunct to endoscopic and histologic surveillance in predicting malignant potential in BE. A recent comprehensive review of investigated and published molecular markers classifies them along the GERD-BE-EAC axis according to their potential usage as either diagnostic tools, indicators of progression, predictors of response to therapy, or aids in prognosis[33]. Of course, some markers span several of these denominations. Most of the hundreds of markers being evaluated are not yet approaching clinical utility, and another recent review article, using the same categories, discusses what requirements remain for clinical implementation of several of the more promising markers, such as larger prospective studies and external validation[31]. Since many of the molecular markers under investigation involve the differential expression of genes from normal to BE to dysplasia to EAC, another way to categorize these approaches could be where they fall along the axis of DNA to RNA to protein. Again, in some cases the same marker may be detected at multiple points along this axis.

Genetic coding

A hereditary component to BE and EAC has long been postulated[34] with reports of familial clustering, but most evidence has favored environmental rather than genetic risk factors[4]. A recent genome-wide association study, using large population-based epidemiological databases, compared patients with EAC to those with BE and normal controls. The authors report extensive polygenic overlap between BE and EAC and interpret this as evidence that the genetic basis for EAC is already present at the development of BE rather than occurring during progression. They identify several loci having strong association with both conditions, namely 19p13 in the oncogene-associated transcription coactivator gene *CRTC1*, 9q22 in esophageal speciation transcription factor gene *BARX1*, 3p14 near esophageal development transcription factor gene *FOXP1*, and 16p24 near the putative tumor suppressor gene *FOXF1*[35]. Further investigation will be needed to examine the clinical utility of genomic investigation as a screening or surveillance tool.

DNA content abnormalities are common among malignancies and preneoplastic states and involve all chromosomes. Several studies have demonstrated that such abnormalities, including aneuploidy, tetraploidy, and loss of heterozygosity at 17p and 9p loci, which affect the tumor suppressors p53 and p16, respectively, occur in EAC and may precede progression to cancer by up to 10 years[6,36]. Impressively, patients with all of these abnormalities in the setting of BE were found in one cohort to have a relative risk of EAC progression of 38.7 compared to patients with BE and none of the DNA abnormalities[36].

Epigenetics: DNA methylation

The role of epigenetics, defined as cellular information other than the DNA sequence itself that is heritable during cell division, in cancer development has been the subject of a growing body of literature since the 1980s[37]. An important epigenetic alteration is DNA methylation, which occurs almost exclusively at CpG nucleotides, found in high numbers in promoter regions, and is involved in the regulation of gene expression and silencing[37,38]. In malignancies, this may involve hypermethylation and consequent transcriptional repression of tumor suppressor genes or hypomethylation and increased expression of oncogenes[39].

Several recent studies have examined the role of DNA methylation in BE and EAC development. A genome-wide profiling, using microarray and hierarchical clustering analysis, of CpG methylation in esophageal tissue samples found that there was substantial difference in methylation pattern between normal esophagus samples and those with BE or EAC, but that the difference between BE and EAC was less clear[38]. This finding also suggests that the epigenetic, as well as genetic, alterations present in EAC may already be present in BE, thus suggesting potential markers for BE surveillance. However, a significant limitation of this study was that all of the BE samples were obtained from patients who developed EAC, as opposed to the vast majority of cases of BE that do not progress[38]. This weakness would become strength if future work demonstrates differences in methylation

patterns of these pre-malignant BE samples from those of nonprogressing, nondysplastic BE.

This was addressed by another recent study, which used DNA methylation arrays to differentiate between BE and EAC in tissue samples. This work delineated four genes (*SLC22A18*, *PIGR*, *GJA2*, and *RIN2*) which, when taken together, had an excellent receiver operating characteristic curve (AUC = 0.988) to distinguish BE from EAC. The authors applied this 4-gene methylation panel to a prospective multicenter study and presented evidence that it can detect nearby dysplasia or early neoplasia in endoscopic biopsies of BE even in the absence of visible histologic change in that particular sample, suggesting a field effect as observed in other types of malignancy. They proposed that patients with BE can be stratified into low, medium, and high risk of malignant progression using this panel as an adjunct to histopathologic evaluation but cautioned that follow-up data on its predictive power is not yet available[40].

Other publications focus on differential methylation of individual genes. As an example, endoglin, or ENG, is a transmembrane glycoprotein with a role in angiogenesis; hypermethylation of its encoding gene's promoter region has been associated with several cancers. Recently, this hypermethylation was found in human esophageal tissue, with frequency of 11.9% in normal esophagus and increasing sequentially to 13.3% in BE, 25% in dysplastic BE, and 26.9% in EAC. However, the frequency of ENG hypermethylation is greater in esophageal squamous cell carcinoma and thus may be more useful as a biomarker for this malignancy[41].

Epigenetics: microRNA

Another active field of research in cancer epigenetic markers is the use of microRNA (miRNA) signatures. MiRNAs are small, non-coding RNAs that regulate RNA translation including that of oncogenes and tumor suppressors; the current state of this research in esophageal cancer has recently been reviewed[42]. Based on analysis of multiple recent studies on miRNA in EAC and BE, the reviewers found that four miRNAs (miR-25, -99a, -133a, and -133b) have potential as diagnostic markers and five (miR-21, -27b, -126, -143, and -145) may have utility as both diagnostic and prognostic markers[42].

Two studies not included in the aforementioned review due to their very recent publication sought to assess the miRNA signature of BE and EAC using microarray analyses and hierarchical clustering, much like the DNA methylation studies described above and with similar results. A genome-wide analysis of miRNA expression levels showed clustering of BE and EAC signatures together as compared with that of normal esophageal tissue but interspersing of BE and EAC signals[43]. However, another study using microarray analysis showed a distinct pattern in EAC, with different patterns of up- and down-regulation seen in EAC compared with BE. This study also showed two miRNAs which were up-regulated in BE tissue adjacent to HGD lesions, again suggestive of a field effect for dysplasia that may be clinically useful alongside histologic surveillance[44].

Protein markers

The vast majority of molecular biomarker research in EAC has focused on differential expression of proteins in esophageal tissue. There are several recent review articles describing the state of this research, including a comprehensive list[33] and additional analysis[31], among others. Several promising and recently investigated classes of markers are described here.

One of the best-described cancer-associated proteins is the tumor suppressor p53. In a recently published large prospective case-control study, aberrant p53 expression by immunohistochemistry of biopsy samples was found to have a higher predictive value for neoplastic progression in BE than histologic diagnosis of LGD with strong inter-observer agreement among scoring pathologists. This association was seen with p53 overexpression, but even more strongly with loss of normal p53 expression[45]. This adds further support to prior studies using p53, including a case-control study which showed that using a combination of aneuploidy and overexpression of transcription factor Ki67 and p53 was predictive of neoplastic progression to HGD or EAC, independent of histology[46]. Another well-known protein in the cancer literature is human epidermal growth factor-2 (HER2), a proto-oncogene notorious for its role in predicting clinically aggressive breast cancers. A recent study using immunohistochemical and fluorescent in-situ hybridization methods on samples from patients with EAC showed a correlation between HER2 expression and p53 overexpression as well as early lesion protrusion[47].

Caudal homeobox transcription factor-2 (Cdx-2) is an intestine-specific transcription factor, but is expressed in BE, activated by acid and bile according to *in vitro* studies. It appears to help direct the development of intestinal metaplasia in BE[5]. Recent histologic and epigenetic research suggests that the encoding gene's promoter region is hypermethylated in HGD and intramucosal EAC; Cdx2 expression was correspondingly downregulated in dysplasia compared with BE metaplasia but restored in poorly differentiated invasive cancer, demonstrating gene silencing memory[48].

Stem cell markers have also received considerable attention as predictors of dysplasia and neoplasia in BE, in light of a newer theory of BE development and progression involving the activation of pluripotent esophageal stem cells to develop intestinal metaplasia in response to gastric acid and bile[5]. Leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5), a downstream target of the Wnt pathway and an intestinal stem cell marker, has been identified in immunohistochemical analyses of BE and shown to have increased expression in HGD and EAC as well as an apparent association with poor survival[49]. Doublecortin and CaM kinase-like-1, also a putative gastrointestinal stem cell marker, similarly has shown a progressive increase in expression from BE to dysplasia

to EAC by immunohistochemistry[50].

Cell signaling to control such processes as proliferation and apoptosis is tightly regulated by receptor tyrosine kinases (RTKs). Disruption of this balance is a common factor in various types of cancer[51]. A recent report showed increased expression and gene copy numbers of tyrosine kinase EPHB4 in both squamous cancer and adenocarcinoma of the esophagus, with corresponding supporting evidence in mouse and cell culture models[52]. Among the RTKs felt to be most promising as markers in EAC are EGFR, ErbB2, ErbB3, FGFR2, and Met, which have been shown to be up-regulated at early stages in dysplasia[53]. However, they have thus far met with mixed results as predictors of malignant progression, perhaps in part due to their heterogeneous expression among individual cancers[54]. A recent study described this heterogeneity using an RTK array; these differentially expressed proteins have great promise in therapeutics as targets of individualized therapy using different tyrosine kinase inhibitors depending on the RTK expressed *in vitro*[54]. For example, antibodies to EGFR and HER2 are promising therapeutic treatments for EACs expressing these particular RTKs[55-58]. The MAPK pathway, downstream of these individually varied RTKs, was frequently activated in pre-malignant and malignant states in human gene expression, representing another potential target for surveillance and treatment[54].

Another class of proteins known to have involvement in malignancy is that of mucins; these secreted and transmembrane glycoproteins function in limiting the activation of inflammatory responses and may become deregulated in states of chronic inflammation, leading to impaired epithelial repair and malignant transformation[59]. Based on initial immunohistochemistry analysis, regulation of different mucin proteins may be involved in BE progression, with decreased expression of the mucin aG1cNAc observed in Barrett's epithelium adjacent to EAC compared with nondysplastic BE when controlled for expression of the scaffold protein MUC6[60].

Literature on the use of NSAIDs including aspirin as therapy for BE has also evolved, and has included the use of prostaglandin E2 (PGE2) as a surrogate endpoint marker. PGE2 is associated with up-regulation of proliferation, resistance to apoptosis, angiogenesis, and increased cellular invasiveness and thus has a theoretically sound basis and utility in these research studies. However, it will need further validation for use as a clinical biomarker[61].

Molecular marker panels and associated conditions

Given the limitations and early phase trials of each of the above and many other candidate molecular markers when assessed alone, it is appealing to consider combining them as a panel and using with other associated risk factors to achieve better predictability of dysplastic progression. For example, given the known association between obesity and EAC has been shown[6], it stands to reason that markers of obesity may be predictive of malignant transformation. Indeed, in patients enrolled in the Seattle BE study (all with BE), increased levels of leptin and insulin resistance were associated with increased EAC risk, while increased high-molecular-weight adiponectin was inversely correlated with EAC[62].

A recent analysis of data from a nested case-control study assessed the utility of a panel of several established biomarkers (abnormal DNA content, p53, and cyclin A expression) and newer biomarkers (levels of sialyl Lewis-a, Lewis-x, and Aspergillus oryzae lectin (AOL) and binding of wheat germ agglutinin) on tissue samples from patients diagnosed with BE who either progressed or did not progress to EAC (cases and controls). A conditional logistic regression analysis was employed, which identified the best panel for risk prediction, consisting of LGD, abnormal DNA ploidy, and AOL. This panel of biomarkers conferred an odds ratio of 3.7 for EAC progression[63].

IMPROVING SAMPLING: NON-ENDOSCOPIC METHODS

A major limitation of the current molecular markers discussed above is that, no matter how sensitive or specific they may be in detecting dysplasia, they depend on adequate tissue sampling by random biopsies. Given the limitations of current endoscopic sampling practices as discussed above, a major remaining challenge is to improve the yield of tissue sampling. One approach relies on "field effect" of malignancies. This refers to the concept that genetic and environmental factors create a broad field of injury, upon which further insult leads to the formation of focal neoplasia[64]. As discussed above, some markers were present not only in areas of dysplasia or neoplasia but also in adjacent tissue, and the majority of genetic and epigenetic abnormalities were found to be already present in pre-dysplastic BE, illustrating this concept. A recent study investigated whether brushings from proximal squamous epithelium in patients with distal EAC exhibited intracellular nanoarchitectural changes as measured by partial wave spectroscopic microscopy, a technology that measures intracellular spatial distribution. Significant differences were observed using this technique, which is encouraging as it could allow for detection of distant malignancy with a minimally invasive approach[65]. However, by the time EAC is present it is often too late to intervene effectively, and it is presently unknown if a similar approach would detect earlier phases of dysplasia.

Several non-endoscopic techniques for screening and surveillance have garnered attention in recent years. One that has shown promise as a potential screening tool in the primary care setting is the Cytosponge. This is a sample acquisition technique in which a pill is swallowed following which a sponge expands in the stomach and is withdrawn *via* the esophagus, brushing off cells in the process. This is safe and well tolerated by patients in initial studies and has diagnostic potential when combined with a potential BE biomarker trefoil factor 3[66]. A microsimulation model predicts that screening 50-year-old

men with GERD using this technology would be cost-effective and reduce mortality[67]. A higher-tech approach to screening and perhaps surveillance is tethered capsule endomicroscopy, in which a pill-sized optical coherence tomography (OCT, see below) probe is swallowed and has the capability to obtain microstructure level imaging of the entire esophagus without requiring sedation[68].

SERUM BIOMARKERS

Although these less-invasive techniques show promise for reducing sampling error and achieving a broader screening population, they do not have the ease of use of a simple blood test. Researchers are working to find a biomarker that is present in the serum that could objectively aid in assessing risk of malignant transformation. Though such a marker has thus far proven elusive, several groups have demonstrated promising findings using antibodies to the well-described tumor protein p53. These antibodies form in response to overexpression of mutant p53 protein in patients with a variety of malignancies and are rare in serum from healthy control patients[69]. A study of serum samples of patients under endoscopic surveillance found a small number of patients who had detectable anti-p53 antibodies in serum samples taken before they were diagnosed with cancer[70]. A meta-analysis of 15 studies found that patients with esophageal cancer were approximately 7 times more likely to have serum p53 antibodies than those without cancer, but the marker was limited by poor and variable sensitivity[71]. A recent case report describes the post-operative surveillance of a patient with EAC over four years, showing lower titers of anti-p53 antibody in the serum after resection and suggesting utility of this marker to detect residual cancer in such patients[72]. These findings support the use of anti-p53 antibodies as a potential surveillance tool in patients with known BE or EAC, but its utility as a screening test in a broader population is not yet clear.

Panels including several biomarkers in combination may prove superior to individual markers alone in screening serum samples. Recently, use of serum biomarker panels was evaluated as a potential screening tool for the presence of BE in a VA population[73]. The best panel in this study included serum levels of several cytokines (IL 12p70, IL6, IL8, IL10), leptin, GERD frequency and duration, age, sex, race, waist-to-hip ratio, and *H. pylori* status. These were combined to give a biomarker risk score, with the highest equal to a 10-fold increase in risk of BE[73].

ENDOSCOPIC IMAGING TECHNIQUES

The mainstay of screening and surveillance of BE is standard white light endoscopy. Particularly with the increased resolution and high-definition monitors in current use, endoscopy is a successful screening modality as it allows for excellent visualization and the ability to sample tissue[6,74]. Dysplasia detection has been shown to increase with longer inspection time in patients with BE[75], a finding with clear relevance to the use of endoscopy as a surveillance tool. However, dependence on endoscopic surveillance with four-quadrant biopsies has to date not been successful in decreasing mortality from EAC and has raised concerns of cost-effectiveness, as mentioned above. Thus, a number of enhancements to conventional endoscopy are being explored to achieve more effective surveillance. An ideal imaging tool would improve objectivity, have a wide area of surveillance, produce results rapidly in real time, and have improved sensitivity and specificity for the detection of dysplasia compared to white light endoscopy. Current modalities in practice and under investigation were recently reviewed[74] and are discussed here.

Chromoendoscopy

The oldest and most "low-tech" of the available endoscopic image enhancements, chromoendoscopy involves the application of stains to mucosal surfaces during endoscopy to enhance visualization of musical surfaces. These stains are characterized as absorptive (*e.g.,* Lugol's iodine, methylene blue, toluidine blue), reactive (Congo read, phenol red), and contrast (indigo carmine)[76]. Methylene blue has been well studied in BE due to its propensity to stain intestinal metaplasia consistent with BE while sparing gastric mucosa, which may be useful for diagnosing short segment BE[74,77]. Widespread use of chromoendoscopy has been limited by variability of staining, laborious effort, and unclear correlation with dysplasia, and there is evidence demonstrating a lack of interobserver agreement or yield identifying early neoplasia in BE with the addition of indigo carmine or acetic acid to white light images[78]. More recent advances in endoscopic imaging have allowed for combination of chromoendoscopy with optical magnification, which has led to descriptions of characteristic relief patterns known as pit patterns[79,80]. While these patterns have shown to have good sensitivity for BE detection, a recent study found them to have low specificity, which may limit their clinical utility in targeting biopsies[81].

Optical enhancements

Improvement in digital endoscope technology has made endoscopic image enhancement possible without the mess of chromoendoscopy, earning the term "virtual chromoendoscopy." Narrow band imaging (NBI, Olympus) uses specific wavelengths of light to construct an enhanced image, and flexible spectral imaging color enhancement (Fujinon) and i-Scan EPKi processor (Pentax) apply digital filters to white light images[74]. NBI has been evaluated in BE. In the same study mentioned above for chromoendoscopy, NBI similarly failed to improve diagnostic yield or interobserver agreement[78]. On the other hand, a recent study demonstrates comparable or improved rates of BE detection but with fewer biopsies compared with standard methods[82], and a meta-analysis demonstrates high accuracy and precision in diagnos-

ing HGD in BE[83]. Thus this modality appears to have potential utility as both a screening and surveillance tool. Taken together, a meta-analysis and systematic review concluded that advanced imaging techniques using chromoendoscopy or virtual chromoendoscopy were found to improve diagnostic yield for dysplasia or cancer in patients with BE compared to white light endoscopy, but there was no significant difference in yield of detection between the two advanced imaging techniques[84].

Autofluorescence and trimodal imaging

Autofluorescence imaging takes advantage of endogenous fluorophores (*e.g.,* collagen, nicotinamide, adenine dinucleotide, flavin, and porphyrins), which can be stimulated by excitation (short-wavelength) light[85]. This has the advantage over white light endoscopy of producing real-time fluorescent images that may aid in detection, but initial systems have been limited by false positives from ulcers and inflammation rather than true dysplasia[85]. More recent efforts have combined autofluorescence with magnification endoscopy and narrow-band imaging ("trimodal imaging"), providing improved visualization of microvascular and microstructural architecture in malignant and premalignant gastrointestinal lesions[86]. Endoscopic trimodal imaging has been shown to be more effective in improving the targeted detection of HGD or EAC in BE[87].

However, this advantage seemed to no longer be present when trimodal imaging was evaluated in a community setting[88].

Fluorescent lectins

A more sophisticated adaptation of chromoendoscopy involves the targeted binding of markers, which are specific to areas of dysplasia. A recent study utilized the alteration in cell-surface glycans over the progression from BE to EAC. A fluorescently-tagged lectin, wheat germ agglutinin, was sprayed over the esophageal mucosa during endoscopy and was found to have specific binding permitting visualization of high-grade dysplastic lesions that were not visible by white light endoscopy alone[89]. This type of molecular imaging has considerable promise as a surveillance tool if findings are borne out in clinical trials.

Confocal laser endomicroscopy

A number of high-tech, high-resolution imaging modalities are currently under investigation. One of these is confocal laser endomicroscopy (CLE), which is in effect an endoscopic light microscope, enabling "optical biopsy" or near-histologic level of detail and tissue enhancement *via* the application of topical or IV contrast agents[74]. Existing commercial CLE systems are endoscope-based (Optiscan, Pentax) or probe-based (Cellvizio)[74]. A multicenter randomized-control trial using probe-based CLE showed significantly improved detection of neoplasia (HGD or EAC) compared with white light endoscopy[90]. Despite high specificity, there has been some concern about sensitivity of this method, which may be related to its limited field of view[91]. Early dysplastic changes are still being characterized, including pit patterns and possible vascular changes, but these remain largely subjective in interpretation. While this technology is promising and may have a role in specialized cases, application of this expensive, time-consuming, and operator-dependent modality in the community is unlikely to occur in the near future.

Optical coherence tomography

Another promising high-tech modality is optical coherence tomography (OCT), which is a high-resolution, cross-sectional imaging technique that utilizes backscattered light waves in a manner analogous to ultrasound with sound waves[92]. It has shown promising accuracy for detection of dysplasia and may help target biopsies[93]. OCT has several advantages as a surveillance tool – it has a wider field of view than confocal microscopy but similar resolution, does not require contrast administration, allows rapid image acquisition and 3-dimensional reconstruction, and can detect subsurface changes. This latter characteristic, the ability to visualize subsurface structures at greater depth than other modalities, enables accurate assessment of BE thickness and presence of SSIM before or after ablation, which in turn correlate with ability to achieve eradication of intestinal metaplasia using RFA[30,94,95]. Like other such modalities, though, OCT is presently costly and operator-dependent and likely has more of a future in tertiary centers. Given less distal optical requirements compared to confocal microendoscopy, however, OCT can be miniaturized for potential non-endoscopic screening of BE, as recently employed using a swallowed tethered capsule[68,96].

Elastic scatter spectroscopy

Elastic scatter spectroscopy (ESS) is related to optical scattering efficiency caused by optical index gradients of cellular and subcellular structures, allowing for detailed evaluation of microstructural features such as nuclear size, crowding and chromaticity, chromatin granularity, and mitochondrial and organellar size and density[97]. This technique has shown promise in preliminary studies, notably decreasing the number of biopsies required to diagnose dysplasia compared to the Seattle protocol[98], but more prospective data is needed.

Angle-resolved low-coherence interferometry

Another novel endoscopic imaging tool is angle-resolved low-coherence interferometry (a/LCI), which uses the distribution of elastically scattered light to make depth-resolved measurements of the size and index of refraction of cell nuclei. In BE, this can be employed to evaluate dysplasia up to significant depth, and preliminary studies indicate that it is accurate in doing so[99,100].

Raman spectroscopy

Finally, a tool that is being developed at present for en-

doscopic use is Raman spectroscopy (ERS), which relies on inelastic light scattering and can assess the biochemical components of its target, notably specific molecular constituents and signals. A recently published study reports high sensitivity and specificity of HGD and EAC detection and the ability to grade dysplasia, as well as the potential to combine ERS with narrow-band imaging for clinical application[101].

FUTURE DIRECTIONS: TOWARD A TARGETED AND OBJECTIVE APPROACH

Recent years have seen considerable research efforts devoted to the development of molecular markers and endoscopic imaging techniques to improve detection rates and diagnostic accuracy for esophageal adenocarcinoma and its premalignant conditions, BE and especially dysplastic BE. A great many molecular markers have been studied and are at varied phases of biomarker development using benchmarks established by the National Cancer Institute. Thus far, no single marker alone has shown sufficient improvement in accuracy of early detection compared with current guideline-based practice to warrant widespread clinical use. Perhaps the greatest promise has been shown by panels of several markers taken along with clinical risk factors and current endoscopic surveillance practices, which can be combined to yield risk scores similar to those used as predictive models in other disease states. Future biomarker research will likely focus on improving the predictive accuracy of these models.

A significant limitation to the ability to reliably detect small early foci of dysplasia on a background of metaplasia is the current reliance on random and limited, rather than targeted, sampling. Even a molecular marker with perfect sensitivity and specificity is only as good as the sample on which it is tested. Thus a major unmet need for improving detection will require improved endoscopic imaging modalities, likely used in combination, to locate such foci of dysplasia. This can be accomplished by improving visualization of the entire mucosal surface, using techniques such as microscopy, chromoendoscopy, optical enhancements, and fluorescence, or by using novel tools like CLE, OCT, ESS, a/LCI, or ERS to obtain an "optical biopsy" of subsurface structure and microstructure. Improvement in surface imaging may require combining imaging techniques, as has been illustrated by trimodal imaging, and further developments will likely validate and improve upon these methods. Subsurface imaging efforts will further confirm the correlations between optical findings and microstructural and biochemical composition. Optimal imaging tools will have the ability to evaluate broad areas of the esophagus, quickly hone in on those areas of highest significance, and have less dependence on subjective analysis when guided by simultaneously applied appropriate biomarkers.

Another way to mitigate the problem of sampling error is to take advantage of the field effect in malignant progression. This principle is relevant both for molecular marker and endoscopic imaging research. The prospect of using non-endoscopic sampling such as sponge or brush methods is appealing as a screening tool, if it can be combined with a sufficiently accurate marker. If field effect can be adequately demonstrated with a given marker on brush or biopsy samples, random sampling would be less troublesome for diagnostic purposes. Advanced optical imaging techniques have been investigated to detect ultrastructural cellular and vascular alterations suggestive of field effect in the colon cancer literature[64], and such efforts will likely be undertaken in the esophagus as well.

Even the most advanced endoscopic imaging techniques suffer from dependence on subjective interpretation by the endoscopist during examination, much as standard histologic evaluation of biopsy samples relies upon subjective determinations by the pathologist. Limiting this subjectivity in histopathology is a key goal of molecular marker development, and similar efforts should also be made in endoscopic imaging. Taking advantage of properties like autofluorescence and specific targeting of molecules to dysplastic foci *in vivo*, it may be possible to combine advanced imaging with molecular markers to achieve this goal. An ideal system would seamlessly integrate a marker of high predictive value with imaging technology allowing for microscopic level imaging of surface and subsurface structure, allowing for objective and targeted diagnosis and therapy.

As systems emerge that reliably demonstrate superiority to conventional approaches in the early detection of dysplasia and EAC, the degree to which they can be reasonably implemented as population-wide surveillance tools will become an important focus of investigation. These techniques require highly trained operators and at present are expensive and not widely available. At the outset, it can be expected that advanced modalities will be effective tools primarily at large academic centers, which may shift the responsibility of BE surveillance toward these institutions. As more providers become trained in the use of these systems and their cost decreases, their use in community settings should become more widespread.

CONCLUSION

Current screening and surveillance methods for the early detection of esophageal adenocarcinoma remain suboptimal given this cancer's increasing incidence and high mortality. Significant challenges include limitations in tissue sampling, lack of objectivity in describing premalignant states, and difficulties in targeting diagnostic and therapeutic modalities. Advances in biomarker development, from genetic and epigenetic characteristics to protein expression profiles, new approaches to sample acquisition, and novel endoscopic imaging tools allowing for improved surface and subsurface visualization, have shown considerable promise in addressing these issues. Future research endeavors will determine which

combination of markers and imaging techniques are most effective in detecting and decreasing mortality from esophageal adenocarcinoma.

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