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Barrett's Esophagus: A Comprehensive and Contemporary Review for Pathologists

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Abstract

This review provides a summary of our current understanding of, and the controversies regarding, the diagnosis, pathogenesis, histopathology, and molecular biology of Barrett's esophagus (BE) and associated neoplasia. Barrett's esophagus is defined as columnar metaplasia of the esophagus. There is worldwide controversy regarding the diagnostic criteria of BE, mainly with regard to the requirement to histologically identify goblet cells in biopsies. Patients with BE are at increased risk for adenocarcinoma which develops via a metaplasia-dysplasia-carcinoma sequence. Surveillance of patients with BE relies heavily on the presence and grade of dysplasia. However, there are significant pathologic limitations and diagnostic variability in evaluating dysplasia, particularly with regard to the more recently recognized unconventional variants. Identification of non-morphology based biomarkers may help risk stratification of BE patients and this is a subject of ongoing research. Due to recent achievements in endoscopic therapy, there has been a major shift in the treatment of BE patients with dysplasia or intramucosal cancer, away from esophagectomy and towards endoscopic mucosal resection and ablation. The pathologic issues related to treatment and its complications are also discussed in this review article.

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INTRODUCTION

Barrett's esophagus (BE) affects 2–7% of adults in Western countries.^{1–3} It is the only recognized precursor of esophageal adenocarcinoma and perhaps a portion of "gastroesophageal junction" (GEJ) adenocarcinomas as well. The annual incidence of esophageal adenocarcinoma in patients with BE has recently been estimated at 0.12–0.13% per year.^{4, 5} Over the last decade, there have been significant advances in our understanding of the biologic and pathologic characteristics of the esophagus and GEJ in response to injury sustained by chronic gastroesophageal reflux disease (GERD). These advancements have led to refinement of our understanding of the pathogenesis of BE and its progression to adenocarcinoma. In this review, we summarize the pathogenetic, biologic, and pathologic features of BE and associated neoplastic lesions both prior to and post treatment, and include discussion of areas that continue to be controversial and/or evolving.

ANATOMY AND HISTOLOGY OF BARRETT'S ESOPHAGUS AND THE GASTROESOPHAGEAL JUNCTION (GEJ)

The esophagus is normally lined by stratified squamous epithelium. Scattered compact submucosal glands and their associated squamous-lined ducts are also normal components of the esophagus. Historically, it was originally believed that the distal 1–2 cm of the anatomic esophagus was normally lined by columnar mucosa, which potentially served as a buffer or transitional zone between the stomach and squamous lined esophagus. However, this concept has now been widely discarded. It is now clear that any type of columnar mucosa located proximal to the anatomic GEJ is metaplastic in origin, and has developed as a result of chronic injury due to GERD. BE represents the end result of metaplastic conversion of normal squamous epithelium of the esophagus to columnar epithelium. Histologically, BE is usually composed of two epithelial compartments, the surface and crypt (or "pit") epithelium, and the underlying glands. There is lack of agreement regarding use of the term "crypt"; or "pit" to describe the functional epithelial unit in BE. This is primarily due to the fact that in BE the functional epithelial unit shares features of gastric foveolar (pit) epithelium and colonic crypt epithelium.^{6, 7} In this review, we use the term "crypt" since in fully developed BE (with goblet cells), this functional epithelial unit shares more features with colonic crypts than it does with gastric pits. However, use of both terms is acceptable in clinical practice. The surface and crypt epithelium in BE is usually composed of a mosaic of cell types, including those normally seen in the stomach (i.e. mucinous cells) and intestine (i.e. goblet cells and less frequently enterocytes, endocrine cells and Paneth cells) (figure 1). In addition, cells with combined gastric and intestinal, or intestinal and squamous features, such as multilayered epithelium, are normally present as well. The proportion of each of these cell types probably depends on the duration and stage of BE development, but the factors responsible for cell differentiation in BE is, essentially, unknown. The glandular compartment, which is located beneath the crypt epithelium, may

be composed of pure mucous glands, pure oxyntic glands, or more commonly, a mixture of both types of glands. The factors that promote gland development are also unknown, but the amount, location, and type of glands is highly variable among BE patients. There is also some evidence that the proportion and type of glands vary depending on the natural history/progression of BE to cancer and the location in the esophagus. For instance, oxyntic glands are more common in the distal esophagus/GEJ region whereas pure mucous glands are more common in proximal BE mucosa. In addition to epithelial changes, BE exhibits mesenchymal and stromal changes, such as duplication of the muscularis mucosae, an increase in the number of blood vessels and lymphatics, and changes in constituent inflammatory cells. The most proximal portion of the stomach is termed "cardia." It is composed of surface foveolar mucinous epithelium and either underlying pure mucous, or mixed mucous and oxyntic glands. The cardia normally transitions to mucosa composed of pure oxyntic glands in the most proximal portion of the gastric body. In some individuals, only oxyntic glands are present at the GEJ ("cardia") so the histologic feature of this small anatomic area is variable. There is ongoing controversy over the origin and nature of "cardiac" mucosa (mucous glands) in the GEJ region in normal individuals (ie. whether it is congenital or metaplastic). Regardless, the length of mucosa composed of mucous glands ranges from 0.1 to 0.5 mm in studies of normal individuals. Several studies have shown that regardless of its origin, the length of mucosa composed of mucous glands in the GEJ region increases with age, and also with the severity of GERD, so that regardless of its origin, in adults, at least a proportion of this type of mucosa is usually considered metaplastic in origin.

Biopsies of the GEJ and proximal stomach usually reveal a mild to moderate amount of chronic inflammation in the lamina propria, and in some cases, neutrophils as well. Mucosal inflammation, regardless of its etiology (GERD vs. *H. pylori* vs. other), is a major underlying stimulus for the development of intestinal metaplasia (IM) in both the esophagus and stomach. Most patients, particularly those without GERD, do not develop IM (goblet cells) in columnar mucosa of the GEJ region. Furthermore, regardless of the presence or absence of goblet cells, these patients are not at significantly increased risk of malignancy. Up to about 30% of patients develop goblet cells in the GEJ region,⁸ but these patients are at very low risk of neoplastic development.⁹ Thus, most authorities do not recommend surveillance of patients with IM in the GEJ region.

DEFINITION AND DIAGNOSTIC CRITERIA OF BARRETT'S ESOPHAGUS

Broadly speaking, BE is defined as columnar metaplasia of the esophagus which is visible endoscopically and confirmed histologically. However, there is controversy with regard to the diagnostic criteria for this disease, and this stems primarily from differences in opinion with regard to the pathologic types of epithelium that result in an increased risk of cancer, as well as other economic and epidemiological issues. For instance, some authorities prefer to define BE according to histologic changes that result in an increased risk of cancer and, thus, a need for surveillance, whereas others use a more pragmatic approach and consider BE as present if the esophagus shows columnar metaplasia (even without goblet cells) regardless of whether the cancer risk is increased significantly. Currently, in the USA, significant cancer risk is attributed only to BE mucosa with IM (defined in most studies by the presence of

goblet cells). However, metaplastic non-goblet columnar mucosa is also at risk for cancer, but the risk is believed to be much lower than columnar mucosa with goblet cells.^{4, 10–12} Regardless of cancer risk, all GI societies, worldwide, require endoscopic identification of columnar mucosa in the esophagus as a necessary prerequisite to diagnose BE. The main differences in criteria concerns the requirement for histologic confirmation (or lack thereof) of goblet cells in biopsies from the esophagus. In a medical position statement on BE, the American Gastroenterological Association (AGA) indicated that, "presently, IM (with goblet cells) is required for the diagnosis of BE because IM is the only type of esophageal columnar epithelium that clearly predisposes to malignancy."¹⁰ In contrast, the updated British Society of Gastroenterology defines BE as "an esophagus in which any portion of the normal distal squamous epithelial lining has been replaced by metaplastic columnar epithelium, which is clearly visible endoscopically (1 cm) above the GEJ and confirmed histopathologically from esophageal biopsies."¹¹ A recent international, multidisciplinary group of GI physicians defined BE "by the endoscopic presence of columnar mucosa of the esophagus," and noted that the pathology report of biopsies of the esophagus should always state whether goblet cells are present in tissue samples obtained from above the GEJ.¹²

Pathologic Interpretation of Goblet Cells

Based on the AGA requirement to identify goblet cells in biopsies of the esophagus in order to diagnose BE in the USA, at this point in time, it is still incumbent on pathologists to perform this task. Unfortunately, there are a number of limitations regarding pathologic interpretation of goblet cells, and these are described further below:

Differentiation of Goblet Cells from Pseudogoblet Cells—Pseudogoblet cells are barrel shaped non-goblet mucinous columnar cells with distended cytoplasmic vacuoles that result in an appearance similar to goblet cells histologically. Pseudogoblet cells contain apical acidic mucin, which may impart a blue hue to the cytoplasm when stained with hematoxylin-eosin, and tend to occur in concentrated rows in the surface and foveolar epithelium. This has led to the synonym "columnar blue cells" or "pseudogoblet" cells. Furthermore, pseudogoblet cells do not contain a triangle-shaped nucleus characteristic of true goblet cells (figure 2). There are no histochemical or immunohistochemical (IHC) stains that can reliably distinguish true goblet cells from pseudogoblet cells. For instance, Alcian blue stains acidic mucin in both true goblet and pseudogoblet cells, although it is typically weaker in the latter.¹³ The level of inter-observer agreement for diagnosing true goblet cells, and distinguishing them from pseudogoblet cells, was extremely poor in a recent study that included seven GI pathologists.¹⁴

Sampling Error—In BE patients, the quantity of goblet cell may vary substantially. The ability to detect goblet cells has been shown to increase proportionally with the number of biopsies obtained at endoscopy, and has been shown to correlate with the length of metaplastic columnar epithelium in the esophagus.^{15–18} For instance, in a study of 1646 biopsies from 125 consecutive patients with endoscopically identified esophageal columnar mucosa, goblet cells were identified in 68% of patients when a mean of 8 biopsies were obtained, compared to 34.7% when a mean of 4 biopsies were obtained.¹⁷ In a study of 3568 biopsies of non-dysplastic columnar-lined esophagus from 1751 patients, Gatenby, et al.

found that the probability of detecting IM (goblet cells) was increased with segment length (10.3% increase per centimeter), and the number of biopsies obtained (24% increase per unit increase in number of tissue pieces).¹⁸

The density of goblet cells, and therefore their detection rate, also varies according to the location in the esophagus.^{19, 20} In one study by Chandrasoma, et al., goblet cells were detected in 100% of BE patients in whom biopsies were obtained from the most proximal aspect of esophageal columnar mucosa, compared to 69% of patients in whom biopsies were obtained from the distal esophagus.¹⁹ However, this is a controversial issue since some studies have, in contrast, reported a mosaic or random distribution of goblet cells within BE.^{15, 21} In a recent study, Theodorou and colleagues reported that the density of goblet cells correlated directly with an esophageal luminal pH gradient, suggesting that goblet cell differentiation is pH dependent and may be due to the effects of pH on bile acid dissociation.²²

Dynamics of Goblet Cell Metaplasia—Within esophageal columnar lined esophagus, goblet cells may fluctuate with progression of disease, time and/or therapy.^{15, 23–25} In a study by Oberg, et al., after 6 endoscopies were performed at intervals of 1 to 2 years, the likelihood of detecting goblet cells in patients with 1–2 cm segments of columnar lined esophagus increased to 63.6% compared to 30.5% at index endoscopy.¹⁵ Similarly, in a study of 43 consecutive patients in whom short-segment BE was suspected endoscopically, but the initial biopsy failed to reveal goblet cells, Jones and colleagues found that biopsies from 10 of those patients (23%) demonstrated goblet cells at the time of repeat endoscopy which was performed within a mean interval of 8.8 months (range: 0.5–31 months) from the first index endoscopy.²³ In a Veterans Administration (VA) study that included esophageal biopsies from patients with esophageal columnar epithelium >3 cm in length, Kim et al. found that 20% of these patients did not exhibit goblet cells after two endoscopies.²⁴ Some studies have reported an association between the presence of goblet cells and male gender, white race and higher patient age.^{16, 18, 26, 27} For instance, pediatric patients with BE often have very few goblet cells, or none at all, in their columnar-lined esophagus.¹⁷

Esophageal Columnar Metaplasia without Goblet Cells

Some patients, particularly those with short or ultra-short BE, either do not have or never develop goblet cells in their columnar-lined esophagus. The risk of cancer in these patients is unknown, and is a source of controversy, since, for decades, most authorities have believed that goblet cells are a surrogate biomarker of epithelium at risk for neoplastic progression. Goblet cells are terminally differentiated non-proliferative cells that secrete mucins that presumably helps protect the mucosa from toxic injury. Although most cancers arise in epithelium with goblet cells, the vast majority of BE patients with goblet cells do not develop cancer. Thus, as a cancer biomarker, goblet cells are highly non-specific. Interestingly, goblet cells have recently been shown to be inversely related to progression of neoplasia. In a recent cohort study of 214 BE patients who were followed for a mean of nearly 8 years, Golden and colleagues reported that the number and proportion of goblet cells were inversely related to the risk of cancer and aneuploidy. The result of this study

suggests that goblet cells may actually be ‘protective’ against progression to cancer, rather than representing biomarkers of cancer progression.²⁸

Esophageal columnar mucosa with goblet cells shows widespread clonal abnormalities and significant alterations in DNA content, even in the absence of morphologically evident dysplastic changes.²⁹ However, some studies suggest that metaplastic columnar epithelium without goblet cells may show similar, or even equal, molecular abnormalities to those that occur in patients with goblet cells.^{18, 30–32} For instances, in a study by Liu et al., 68 patients with BE were analyzed for DNA content by image cytometry and high fidelity histograms. Interestingly, equal DNA content abnormalities were present in metaplastic columnar epithelium with goblet cells compared to epithelium without goblet cells.³⁰ In another study by Chaves et al., DNA abnormalities, in the form of LOH of chromosomes 7 and 18, were seen more frequently in metaplastic columnar epithelium without goblet cells compared to goblet cell containing epithelium.³¹

Several studies also suggest that metaplastic non-goblet columnar epithelium is at risk for progression to adenocarcinoma.^{18, 32, 33} For instance, in a study of 712 patients with esophageal metaplastic columnar epithelium, Kelty and colleagues reported that the incidence of adenocarcinoma in patients with goblet cells was similar to the rate in patients without goblet cells (4.5% vs 3.6%, respectively).³² In another retrospective study of 141 patients with early adenocarcinoma of the esophagus, Takubo et al. showed that the majority of early cancers arose in patients with columnar mucosa devoid of goblet cells.³³ Although these studies provide some evidence to the neoplastic potential of non-goblet columnar epithelium, many of them are retrospective in design and suffer from sampling limitations.

According to more recent large population-based studies, patients with metaplastic non-goblet columnar epithelium have a lower risk of cancer progression than patients with goblet cells, but the risk is greater than the general population.^{9, 34, 35} In a study of 8522 patients with BE, Bhat S, et al reported that the cancer risk was 0.07% in patients without goblet cells, compared to 0.38% in patients with goblet cells at index biopsy ($p < 0.001$).⁴ In another study by Chandrasoma et al., 214 patients who had endoscopic evidence of columnar-lined epithelium and had undergone systemic 4 quadrant biopsies at 1–2 cm interval were evaluated. The result of this study showed that dysplasia and/or adenocarcinoma was only seen in patients with IM, and when this feature was absent, the patient was at no, or extremely low, risk for dysplasia and cancer.³⁴ Westerhoff et al., similarly found dysplasia and cancer only in patients with documented goblet cells, and also noted that patients with short-segment columnar metaplasia without goblet cells did not demonstrate goblet cells on subsequent biopsies, implying that these biopsies may have been obtained from the proximal stomach rather than from BE.³⁵

Even if goblet cells are present, recent data shows that the risk of cancer among patients with short-segment BE is significantly lower compared to those with long-segment BE. In a recent meta-analysis of the incidence of BE associated adenocarcinoma, the annual incidence among patients with non-dysplastic short-segment BE was estimated to be 0.19%, compared to 0.33% overall.⁹ Of course, regardless of the published data, anecdotal observations have clearly documented evidence of early, and definite, dysplastic lesions and

carcinoma in BE patients without goblet cells in their esophageal columnar-lined mucosa. Furthermore, there is some evidence that a significant proportion of "GEJ" cancers actually arise in patients with ultrashort-segments of BE, a condition known to be associated with a low incidence rate of goblet cell metaplasia. The AGA maintains that, presently, there are insufficient data to make meaningful recommendations regarding management of patients who have "cardia-type epithelium" in the esophagus, and does not recommend use of the term "BE" for these patients. Based on this lack of data, the AGA currently maintains that it is justified not to perform endoscopic surveillance for patients with columnar metaplasia without goblet cells.¹⁰

EVALUATION OF GASTROESOPHAGEAL JUNCTION (GEJ) BIOPSIES AND DIFFERENTIATING ESOPHAGEAL METAPLASTIC COLUMNAR EPITHELIUM FROM GASTRIC CARDIA COLUMNAR EPITHELIUM

Pathologists are often asked to evaluate "GEJ" biopsies in order to "rule out BE" in patients who have been found to have an "irregular" endoscopic Z-line (squamocolumnar junction) concerning for ultrashort-segment BE, at the time of endoscopy. In most cases, it is not possible for pathologists to accurately determine the true anatomic location of the biopsy (i.e. whether it was obtained from the distal esophagus or the proximal stomach) when evaluating biopsies from the GEJ region. Goblet cells may develop in both locations, and when present, are histologically and histochemically identical regardless of their site of occurrence. However, some studies have identified a variety of morphologic features that, when present, help determine if a biopsy with columnar mucosa from the GEJ was obtained from the distal esophagus. For instance, in a study by Srivastava et al., mucosal biopsy samples from 20 patients with BE and 20 patients with IM of the proximal stomach were evaluated. They found that the presence of one or more of the following morphologic features, such as squamous epithelium overlying crypts (buried columnar epithelium), severe diffuse crypt atrophy and disarray, multilayered epithelium, and esophageal glands and/or ducts, were indicative of esophageal origin of the columnar mucosa in the biopsy sample, and thus, were significantly associated with BE (figure 3 and table 1).³⁶ Unfortunately, mucin-histochemical or intestine-specific biomarker stains are not useful in this differential either. Markers such as DAS1, CDX2, Hep Par 1, villin, CK7/20, or any of the MUC molecules that are known to be specific for intestinal columnar epithelium, are equally common or not specific to columnar epithelium of the distal esophagus compared to the proximal stomach.³⁷⁻⁴¹ Thus, close interaction between gastroenterologists and pathologists is crucial when faced with patients who may have short- or ultrashort-segments of BE, and in which the biopsy was obtained from the GEJ area. When clinicians are faced with a patient with an irregular Z-line and the possibility of ultra-short segment BE, biopsies of the stomach may also aid in determining if the GEJ biopsy (with goblet cells) is indicative of BE. For example, documentation of chronic gastritis with IM in the stomach would present evidence that goblet cells identified in a GEJ biopsy may be secondary to diffuse chronic gastritis rather than reflux.

PATHOGENESIS OF BE

Presumably, Barrett's metaplasia results from cellular reprogramming in which the expression of key developmental transcription factors is altered in a way that changes the cell's phenotypic commitment.⁴² It has been proposed that progenitor cells may originate in either the esophagus, the proximal stomach, the bone marrow or a combination of these organs. Transdifferentiation is a process in which a fully differentiated cell type (i.e. squamous) changes directly into another (i.e. columnar).⁴³ Transdifferentiation involves individual cells, and can be distinguished conceptually from metaplasia, which involves a change from one type of tissue comprised of multiple differentiated cell types into another tissue type.⁴³ In transdifferentiation, the cell changes its phenotype into another of a kind that was present in the organ during embryonic development,⁴⁴ and observations in embryonic mice have suggested a potential role for transdifferentiation in the development of BE. Direct transdifferentiation is a reprogramming event that does not require the cell to divide in order to change its phenotype, and that appears to occur in a number of organs.^{43, 45–47}

There is some histologic evidence that transdifferentiation may occur in BE as well. Biopsy specimens obtained at the squamocolumnar junction in BE's patients often show a type of multilayered epithelium characterized by the presence of squamous cells covered by columnar cells. Some cells within multilayered epithelium display IHC features of both squamous and columnar cells.⁴⁸ Scanning electron microscopy studies have also demonstrated a "distinctive cell" at the squamocolumnar junction with ultrastructural characteristics of both squamous and columnar cells.⁴⁹

Regardless, there is recent evidence that metaplasia arises from undifferentiated progenitor cells that have the capacity to produce, and maintain, multiple cell types.⁴³ As a result of GERD-induced tissue damage, immature progenitor cells are reprogrammed to express columnar, rather than squamous, developmental transcription factors. This reprogramming process is termed "transcommitment". Embryogenesis of the mouse esophagus supports this concept.^{43, 45} The embryonic mouse esophagus is lined by columnar epithelium that expresses Sonic hedgehog (Shh), which stimulates esophageal stromal cells to express bone morphogenetic factor 4 (BMP-4). Stromal BMP-4 signals back to the epithelium, where it functions to maintain a columnar phenotype.^{50, 51} Shortly after birth, when Hedgehog signaling ceases and stromal BMP-4 levels drop in the esophagus, columnar epithelium changes into stratified squamous epithelium.^{51–54} When mice are genetically engineered to maintain esophageal epithelial BMP signaling, the esophageal columnar lining persists.⁵⁵ These observations raise the possibility that reactivation of Hedgehog signaling and/or an increase in BMP-4 levels may cause esophageal squamous cells to re-program to a columnar phenotype via transcommitment. In further support of transcommitment, esophageal squamous cell lines and tissues exposed to acid and bile salts *in vitro*, or GERD *in vivo*, show increased expression of transcription factors SOX9 and FOXA2, both of which are targets of the Hedgehog pathway and involved in the development of a columnar phenotype, and of CDX2, which is involved in the development of an intestinal phenotype (figure 4).^{50, 56–60}

One potential esophageal resident progenitor cell population is located in the interpapillary zone of the basal layer of squamous epithelium.^{61, 62} Another potential esophageal resident progenitor cell may be located in the ducts of the submucosal glands.^{63, 64} Regardless, recent research using mouse models has provided strong support for the presence of immature progenitor cells in the proximal stomach.^{65, 66} For instance, Lineage tracing experiments have revealed that metaplastic columnar epithelium develops from Lgr5+ progenitor cells located in the gastric cardia.⁶⁶

Finally, there is some evidence that circulating, multipotential progenitor cells from the bone marrow may also contribute to the development of BE.^{67, 68} In support of this concept, a clinical report of a male patient, who developed esophageal adenocarcinoma after receiving a bone marrow transplant from a woman, described the results of X/Y fluorescence in situ hybridization (FISH) analysis of the resected esophagus, which revealed that approximately 30% of the tumor cells were derived from the female donor.⁶⁸

Once columnar epithelium has developed in the esophagus, there is some evidence to suggest that gastric type mucinous columnar epithelium develops first, and then this epithelium undergoes additional reprogramming which ultimately results in the development of intestinal differentiation and, finally, goblet cells.^{69–72} As mentioned above, when squamous epithelium is exposed to acid and bile acids, inflammation and tissue injury activates signaling pathways such as Hedgehog, BMP4, and NF- κ B, and down-regulates Notch signaling. These signals lead to increased expression of Sox9, which induces columnar differentiation, and FOXA2, Cdx1 and Cdx2, which induces intestinal differentiation.^{73, 74} Several investigators have shown that non-goblet epithelium shows biochemical evidence of intestinalization prior to the morphologic appearance of goblet cells. CDX2 plays an early role, whereas MUC2 a late role, in the intestinalization process.^{63, 71, 73, 74}

PATHOLOGY OF DYSPLASIA IN BARRETT'S ESOPHAGUS

Adenocarcinoma in BE develops via a progressive sequence of histologic and molecular events that begins with metaplasia, then progresses through various stages of dysplasia prior to development of adenocarcinoma. Therefore, routine endoscopic surveillance, followed by histologic assessment of mucosal biopsies for the type and grade of dysplasia is the principle method of risk assessment in patients with BE.¹⁰

Morphologically, dysplasia is defined as neoplastic epithelium that is confined to the basement membrane. There are several types of dysplasia in BE. Some subtypes, such as gastric (foveolar) and serrated dysplasia, have been described only recently and are not as well characterized as intestinal-dysplasia. Occasionally, dysplasia may not show any evidence of differentiation (neither intestinal, gastric or serrated), and conversely, many patients may show a mixture of several types of dysplasia. Regardless of the type, dysplasia in BE is graded as either negative, low, or high grade. "Negative" implies regenerating, but non-neoplastic, epithelium. When a definite diagnosis cannot be established, the term "indefinite for dysplasia" is used. The details of the classification system and criteria used to define dysplasia are discussed in the following sections and summarized in table 2.

Intestinal Type Dysplasia

Morphologically, intestinal type dysplasia (figure 5) resembles epithelium of a conventional colonic adenoma since it is composed of columnar cells with intestinal differentiation, and goblet cells. It is the most common type of dysplasia in BE. Low grade dysplasia (LGD) is characterized by cells with nuclear enlargement, elongation, hyperchromasia and stratification, but with retained nuclear polarity (long axis of the nucleus is oriented perpendicular to the basement membrane). The dysplastic crypts show little, if any, architectural abnormalities and retain visible evidence of lamina propria between the dysplastic crypts. The nuclei may be 2–3× the size of lymphocytes, and may show slight loss of polarity, and piling up on the surface of the mucosa, but usually not in the deep crypts. Mitoses are generally increased in number, more frequent in the crypts compared to the surface epithelium, and typically normal in appearance. The cytoplasm is generally mucin depleted and shows eosinophilia. Goblet cell numbers may vary from numerous to markedly decreased. The most severe changes are usually evident in the bases of the crypts, where dysplasia originates. Thus, in the bases of the crypts, the nuclei may be 3–4× the size of lymphocytes, and show some degree of nuclear variability, pleomorphism and loss of polarity. In some cases, the bases of the crypts may appear crowded, but complex budding or angulation is not a feature of LGD. In general, LGD is similar architecturally and cytologically to the features of a low-grade tubular adenoma of the colon.

In contrast, high grade dysplasia (HGD) exhibits a greater degree of cytologic atypia, but usually also contains architectural abnormalities. Cytologically, HGD shows larger sized nuclei (3–4× the size of lymphocytes), marked nuclear pleomorphism often with round vesicular nuclei and prominent nucleoli, more apparent loss of nuclear polarity, and more frequent mitotic figures, many of which may be atypical and involve the surface epithelium as well. These cytologic features involve not just the bases of the crypts, but usually the full length of the crypts and surface epithelium. The nuclei may retain their elongated "pencil" shape, but if so, they reveal stratification to the surface of the cytoplasm, leaving little or no mucin cap, or visible cytoplasm at the most luminal aspect of the cell. Almost every involved crypt will typically show increased mitoses, equally frequent in the crypts and surface epithelium. In more advanced cases, the nuclei lose their elongated "pencil" shape and instead, show round or angulated nuclear contours, separation from the basement membrane and loss of polarity. In these cases, prominent nucleoli may be present and there may be significant variation in size and shape of nuclei even between cells of individual crypts. In some cases, HGD is diagnosed on the basis of these cytologic features alone. However, in most cases, the epithelium shows significant architectural abnormalities as well, such as irregular size and shape of crypts, crowded (back to back) crypts, with little or no intervening lamina propria, intraluminal budding or cribriforming, villiform surface configuration, and dilated glands with intraluminal necrotic debris. If one or more these architectural features are present, but the nuclei appear low-grade, the lesion is still considered "high-grade".

Low vs High-Grade Dysplasia—A common problem when evaluating biopsies for the degree of dysplasia is in determining how many HGD crypts are needed to be present in order to upgrade a predominantly low-grade lesion to high-grade. Unfortunately, there are no

published guidelines on this topic. Presumably, the degree of dysplasia is proportional to the risk of neoplastic progression. In one long term outcome study that evaluated the prognostic value of extent of dysplasia (total), and of LGD and HGD separately, from 250 BE patients with dysplasia who were followed for progression to cancer, both the total extent of all dysplasia, and the total extent of LGD in particular, were significant predictors of cancer development, but the extent of HGD alone was not.⁷⁵ That study suggested that once HGD develops (to any degree), the patient has an elevated risk of cancer. Nevertheless, based on this limited data, we believe it is justified to establish a diagnosis of HGD if any amount of HGD (even one crypt) is present in the biopsy samples. Use of a standardized reporting form is recommended in the setting of BE to improve completeness, accuracy and reproducibility of the morphologic findings.¹²

Early (“Crypt”) Dysplasia—Regardless of the grade, dysplastic epithelium usually involves both the crypt and surface epithelium. In fact, it is the finding of surface involvement that, in many cases, helps pathologists distinguish true dysplasia from regenerating crypts. However, several studies have shown that dysplasia develops initially in the bases of the crypts, presumably from multipotential stem cells, and progresses, with time, to involve the upper crypts and surface epithelium.^{6, 76–83} Thus, on occasion, dysplasia may be detected at an early stage of development when it involves only the crypts, without surface involvement (figure 6). This is referred to as “crypt dysplasia.” Although in most cases of crypt dysplasia, the nuclear changes are low-grade, rarely, high-grade nuclear changes may involve only the crypt bases as well. Historically, pathologists have categorized this stage of neoplasia as either “indefinite for dysplasia” or “negative for dysplasia,” based on the erroneous belief that if epithelium matures to the surface, it cannot be neoplastic. However, neoplastic epithelium can mature and appear non-dysplastic at the surface, similar in essence, to sessile serrated adenoma/polyp of the colon, or squamous dysplasia of the esophagus.⁸⁴ In fact, this phenomenon occurs in the stomach as well in patients at risk for gastric cancer, and is referred to as “pit” dysplasia in that circumstance.^{85–87} One study, by Lomo et al, showed that 87% of patients with crypt dysplasia (all low-grade) showed conventional (full crypt and surface) dysplasia elsewhere in their esophagus. Based on this data, the authors concluded that the finding of crypt dysplasia in a biopsy should alert the pathologist that there is a high likelihood of finding conventional dysplasia in other biopsies from the patient, and, thus, if the latter is not seen on initial levels, further deeper levels should be performed.⁷⁷ Several outcome studies of crypt dysplasia have been performed.^{79, 81, 83} In a recent study, the outcome of 12 patients with low-grade crypt dysplasia, but without synchronous conventional LGD or HGD, from a cohort of 214 high risk BE patients, were followed for a mean of 90.4 months (range: 2.3–176 months).⁷⁹ Seventeen percent of patients with crypt dysplasia developed conventional LGD, 17% developed conventional HGD, and 8% developed adenocarcinoma. In another recent outcome study of patients with crypt dysplasia, (mostly low-grade) 26% progressed to HGD or carcinoma, which was similar to a comparative group of patients with conventional LGD.⁸³ From a pathologist's point of view, crypt dysplasia should be diagnosed on the basis of the grade of nuclear (cytologic) changes, since at this stage, architectural features of the crypts are typically well preserved. The mechanism of dysplastic crypt expansion is unknown, but there is evidence to suggest bidirectional movement of dysplastic cells within

BE's epithelium, and expansion by budding and proliferation of the deep aspects of the crypts into the lamina propria.^{64, 80, 88} Diagnosing crypt dysplasia in the presence of active inflammation and/or ulceration is extremely difficult and, thus, should be avoided.

Unconventional Dysplasia

Some types of BE-associated dysplasia do not show cytologic features of intestinal differentiation. Instead, they show mucinous ("foveolar") cytoplasmic changes, showing either few, or a complete absence of, goblet cells, reminiscent of gastric foveolar epithelium.^{89–94} Non-intestinal ("foveolar") dysplasia has been reported to account for 6–8% of all BE-associated dysplasia cases, but true prevalence studies have not been performed.

Morphologically, foveolar dysplasia shows epithelium composed of cells with abundant mucinous cytoplasm and few, if any, goblet cells. Cytologically, the cells have a uniform, usually single layer of small or slightly enlarged, round to oval-shaped and basally located, nuclei, without stratification or significant nuclear pleomorphism (figure 7). In this type of dysplasia, the surface epithelium is always involved, and paradoxically, the bases of the crypts may, in fact, be spared. When low-grade, foveolar dysplasia may be difficult to recognize and distinguish from non-neoplastic, gastric cardia mucosa, particularly when the latter is inflamed.⁹² Mitoses are usually rare, and goblet cells and Paneth cells are typically absent.^{90–92} In fact, anecdotal data suggests that this type of dysplasia may occur more frequently in metaplastic non-goblet columnar mucosa in the esophagus.⁹³ At least one study has shown an association with adenocarcinomas with gastric differentiation, rather than intestinal differentiation.⁹⁴ Thus, some authorities have proposed that there are, at minimum, two distinct mechanisms of cancer development in BE, one with an intestinal precursor and one with a gastric epithelium precursor.^{94–96} In a recent study of 156 patients that underwent resection of BE associated adenocarcinoma, Agoston et al. found that 122 patients (78%) showed dysplasia in background mucosa and that, in general, the type of dysplasia (i.e. intestinal, foveolar or mixed) correlated with the type of adenocarcinoma that was present in the esophagus.⁹⁴

Given that the dysplasia classification system originally proposed for BE did not recognize this distinct type of dysplasia, features to distinguish low from high-grade foveolar dysplasia have not been formally published. Nevertheless, our personal experience indicates that similar to intestinal dysplasia, a spectrum of cytologic and architectural abnormalities may arise in foveolar dysplasia as well. High-grade changes are characterized, cytologically, by enlargement of round to slightly oval shaped nuclei with a more open chromatin pattern and prominent nucleoli, and increased mitoses. However, significant loss of polarity, stratification, and pleomorphism are not typical features of foveolar HGD. In fact, even in HGD, the cells often retain fairly regular appearance. However, architecturally, the crypts are usually more compact, elongated and show extensive branching and complexity without intervening lamina propria. Most striking is the presence of a marked increase in the N/C ratio of the cells due to a combination of large sized nuclei and mucin depleted cytoplasm.

Another rare form of unconventional dysplasia is serrated dysplasia (figure 7). Serrated dysplasia is a form of "intestinal" dysplasia since it is composed of epithelium with

intestinal differentiation and goblet cells, but the pattern of growth is distinct from conventional intestinal-type dysplasia. Similar to foveolar dysplasia, the morphologic, biologic features, and malignant potential of serrated dysplasia, have not been well characterized. Low-grade changes resemble cytologic and architectural features of a serrated adenoma of the colon, being comprised of cells with small oval-shaped hyperchromatic nuclei with abundant hypereosinophilic cytoplasm arranged in a luminal saw-toothed, or serrated, growth pattern. Goblet cells may be present, but are usually few in number. Mitoses are generally infrequent. Similar to foveolar dysplasia, the most involved areas may, in fact, be the surface epithelium and superficial crypts, rather than the crypt bases. HGD changes show larger and usually more irregular shaped nuclei, increased stratification, increased mitoses, more significant loss of polarity and, typically, a prominent intraluminal budding and hyper-serrated pattern of growth. HGD usually involves all levels of the crypt and surface epithelium.⁹³

The natural history of foveolar and serrated dysplasia is poorly understood. Some studies have shown that these subtypes of dysplasia may have a more aggressive growth rate and/or natural history than conventional intestinal LGD, and are more similar to intestinal HGD.^{89, 93} In one study of 18 BE patients with non-intestinal (“foveolar”) dysplasia from a cohort of 270 high risk BE patients, patients with non-intestinal dysplasia showed a high association with HGD of intestinal type elsewhere in the esophagus, and also showed a significantly higher rate of DNA flow cytometric abnormalities.⁸⁹ In a recent follow-up study, the morphologic, DNA flow cytometric, and outcome features of 17 BE patients with foveolar dysplasia, and six patients with serrated dysplasia, were evaluated and compared to BE patients without dysplasia.⁹³ In that study, flow abnormalities were present in 76.5% of cases with foveolar dysplasia, and both the type and frequency of flow abnormalities and the rate of progression to cancer were similar to conventional intestinal HGD.

Limitations of Grading Dysplasia in BE and Interobserver Variability

Unfortunately, there are several limitations to the BE dysplasia grading system that, in many ways, limits its value clinically. Limitations include: 1) differences in interpretation between Western and Eastern pathologists, 2) use of non-scientifically validated morphologic features to distinguish low from HGD, 3) the confounding effects of inflammation and ulceration which induces regenerative changes so extreme that they mimic dysplasia (both low and high-grade), 4) interobserver and intraobserver variability in interpretation of cytologic and architectural features, 5) difficulties in separating LGD from HGD (as discussed above) and, in particular, HGD from carcinoma (see further below), and 6) lack of recognition and difficulties in diagnosing less common unconventional subtypes of dysplasia, as discussed above. A full discussion of all these items is beyond the scope of this review, but in the following section, we outline various reasons why pathologists may not be able to diagnose dysplasia reliably and, thus, may choose to use the term “indefinite for dysplasia.”

When biopsies contain active inflammation, ulceration or post-ulcer healing, it may be difficult for pathologists to determine if a biopsy with atypical changes represents true dysplasia or simply the extreme of regeneration (or in some cases, degeneration). In these situations, the term “indefinite for dysplasia” may be used as an interim diagnosis.

Pathologists' uncertainty may be due to a variety of reasons. For example, in the presence of active inflammation, erosion or ulceration, regenerating epithelium may show nuclear hyperchromasia, stratification, enlargement and increased mitoses, all features that are also indicative of potential dysplasia. In other circumstances, biopsies may contain a technical or processing artifact (such as thick sectioning, crush artifact, poor orientation or lack of surface epithelium) which precludes accurate assessment of dysplasia. In clinical practice, the rate at which this diagnosis is used varies widely between pathologists, largely based on the degree of individual experience with these lesions.⁹⁷ It is important to recognize that the "indefinite for dysplasia" category is not a distinct neoplasia category. It is only a provisional diagnosis to be used in specific situations of diagnostic uncertainty. For cases diagnosed as such, close interaction between the clinician and pathologist is recommended so that both health care providers have a clear understanding of the precise reason for diagnostic uncertainty, in order to plan further management accordingly. For example, cases in which a diagnosis of "indefinite for dysplasia" is rendered due to the presence of active inflammation or ulceration, a repeat biopsy is recommended within a 3–6 month period after an aggressive anti-reflux treatment has been implemented. In contrast, an immediate re-biopsy may be indicated in cases in which a diagnosis of "indefinite for dysplasia" is rendered because of technical or processing artifact.

There is a well-established high degree of inter-observer (and even intra-observer) variability in the diagnosis and grading of dysplasia in BE among both general and GI pathologists.^{97, 98} In a recent inter-observer study among expert GI pathologists, inter-observer agreement for diagnosis and grading early and late dysplastic lesions in BE was only moderate.⁸² Distinguishing HGD from intramucosal adenocarcinoma is particularly problematic.^{97–99} Pathologists from the USA, Europe and Japan often disagree on the criteria used to distinguish HGD from early adenocarcinoma. For instance, Japanese pathologists place a lot of emphasis on the cytologic abnormalities of cells in order to diagnose carcinoma, whereas most western pathologists usually require evidence of invasion into the lamina propria in order to render a diagnosis of "carcinoma".^{100, 101} While western pathologists widely accept the definition of intramucosal adenocarcinoma as a lesion in which the neoplastic cells have penetrated the basement membrane and invaded the lamina propria, but have not yet passed through the muscularis mucosae, there are no reliable or validated criteria for lamina propria invasion.

Ancillary Techniques for Diagnosing Dysplasia

Given the limitations of morphologic assessment, non-morphology based markers to detect dysplasia in BE are a subject of ongoing research. Several adjunctive diagnostic markers have been investigated which include, but are not limited to, surface expression of cyclin A by IHC, proliferation markers such as Ki67, DNA content (aneuploidy/tetraploidy), telomerase, genetic mutations (p53, p16, Kras, APC, B catenin), growth factors, apoptosis inhibitors, cyclooxygenase 2, and alpha-methylacyl-CoA racemase (AMACR) IHC.¹⁰²

IHC staining for p53 has been the most extensively studied marker. P53 is a transcription factor expressed from the tumor suppressor gene TP53 (chromosome 17p). Inactivating mutations of the p53 gene can be detected by IHC, which shows either complete loss (absent

protein and negative staining), or more commonly, increased expression and positive staining (due to mutations creating a protein product that is resistant to degradation). The frequency of p53 mutation increases in BE neoplasia.^{11, 97, 103, 104} In a study of 53 cases of BE, Younes et al. found that p53 staining occurred in 0% of cases with no dysplasia, 9% of those with LGD, 55% of HGD and 87% of adenocarcinomas.¹⁰⁵ However, p53 IHC suffers from a high rate of false positivity and false negativity. Some studies have shown p53 staining in up to 10% of cases considered morphologically negative for dysplasia.^{105–107} Given the variable results in the literature, the use of p53 IHC to diagnose dysplasia in clinical practice is currently controversial, and thus, most authorities do not advocate its use in this regard.

AMACR IHC has also been investigated as a marker of BE-associated neoplasia.^{108–110} In a study of 101 BE cases, AMACR IHC was positive in 0% of non-dysplastic cases, 22% of cases for indefinite for dysplasia, 18% of LGD, 60% of HGD and 67% of adenocarcinomas.¹¹¹ In a study of 77 specimens from 29 BE patients with adenocarcinoma treated with surgery, 91% of cases with LGD and 96% of cases with HGD /early adenocarcinoma were positive for AMACR.¹¹²

Regardless, at present, morphologic assessment of dysplasia remains the gold standard for evaluating dysplasia. In their most recent position statement, the AGA does not recommend the use of molecular biomarkers to confirm a histologic diagnosis of dysplasia for patients with BE at this time.¹⁰

Natural History of BE-Associated Dysplasia

Estimates of cancer occurrence in patients with low or HGD, respectively, vary from 3%–23% to 4–55% after 5 years of follow-up.^{113–116} The wide variability in reported rates of progression to cancer is attributable to many factors, such as differences in patient populations studied, differences in the proportion of prevalent versus incident dysplasia in the study cohorts, variability in the frequency of surveillance protocols, and pathologist's diagnostic variability, among others.

HGD in BE is associated with, at least, 6% per year incidence of cancer.¹¹⁷ The biologic potential and natural history of LGD is more variable, and, as a result, the methods of treatment are more variable and controversial. For instance, in a recent study by Sharma et al, 618 patients were followed for a combined total of 2456 patients year, and a mean follow up of 4.1 years. In that study, only 12 patients developed cancer. Of the 156 patients with LGD, 66% revealed no evidence of dysplasia upon follow up, 21% showed persistent LGD, and 13% showed progression to HGD or cancer.¹¹⁴ A recent systematic meta-analysis review estimated the incidence of progression of LGD to HGD or adenocarcinoma to be 0–54–1.73% per year.¹¹⁸ In a recent population-based European study, the risk of progression to adenocarcinoma was five times higher in patients with LGD compared to those with no dysplasia.¹¹⁹

Interestingly, several studies have shown that progression rates of low to HGD, and dysplasia to carcinoma, are directly proportional to the number of pathologists who agree on the dysplasia diagnosis.^{97–99, 120} This perhaps relates to the fact that as more pathologists

agree with a particular dysplasia grade, the certainty of that diagnosis rises, and as a result, fewer non-dysplasia cases end up being included in the analysis which tends to eliminate the BE patients who do not progress, or progress at a much slower rate.

In general, there is a tendency among general pathologists to overdiagnose dysplasia. Thus, the rate of progression of LGD is generally higher when it is diagnosed by an experienced GI pathologist compared to a general pathologist.^{75, 121–123} A recent European study found that of 147 patients diagnosed with LGD in the community, 85% of the patients were downgraded to a diagnosis of no dysplasia or indefinite for dysplasia, when reviewed by two GI pathologists with experience in BE-related neoplasia. The progression rate to HGD / adenocarcinoma was 13.5% per patient per year in the confirmed patients, compared to 0.49% in those whose diagnosis was downgraded.¹²¹ Therefore, as mentioned above, a consensus diagnosis by at least 2 pathologists, with at least one who has expertise in GI/BE dysplasia, improves the risk assessment of patients with BE and emphasizes the importance of the AGA and American College of Gastroenterology (ACG) recommendations that all dysplasia diagnoses be confirmed by an expert GI pathologist prior to patient management.¹²⁴

MOLECULAR BIOLOGY OF PROGRESSION OF BE TO ADENOCARCINOMA

This section will briefly review how benign Barrett's metaplastic cells acquire the physiological hallmarks of cancer during the process of carcinogenesis.¹²⁵ Some of the major reported genetic alterations are shown in figure 8, however, the reader should appreciate that these represent only a fraction of the changes required for a benign cell to progress to cancer.

C-myc and Cyclins D1, E, and B have been implicated as oncogenes in BE by allowing the cells to hyperproliferate (Reviewed in ¹²⁶). Barrett's cells may also proliferate without exogenous stimulation by altering growth factors, growth factor receptors and pro-proliferative signal transduction pathways, such as the Ras/Raf/mitogen activated protein kinase (MAPK) pathway.^{127, 128} Recently, the phosphatidylinositol 3-kinase (PI3K) pathway has been identified by exome and whole-genome sequencing as the most frequently altered oncogenic pathway affected by mutation in esophageal adenocarcinomas.¹²⁹ Although the role of *erbB-2* (also called HER2 or Neu) remains controversial, *erbB-2* mutations have been detected in esophageal adenocarcinomas by exome and whole-genome sequencing.¹²⁹

Inactivation of the tumor suppressor genes p53, p16, p15, p27, and adenomatous polyposis coli (APC), have been implicated in BE's carcinogenesis (Reviewed in ¹²⁶). In addition, recent studies have shown that expression of genes, including tumor suppressors, can be repressed by microRNAs (miRNAs), which are non-coding RNA molecules comprised of 18 to 25 nucleotides.¹³⁰ Unique miRNA expression profiles have been found to characterize the progression from esophageal squamous mucosa to Barrett's metaplasia, and from Barrett's metaplasia to adenocarcinoma.¹³¹

The expression of death receptors that are members of the tumor necrosis factor (TNF)-receptor superfamily, and the Fas receptor-Fas ligand pair have been investigated in BE's

carcinogenesis, as well.^{132–134} Enhanced expression of TNFR1 and its ligand, TNF- α , have been found in the progression from Barrett's metaplasia to adenocarcinoma.¹³² Contradictory data exists on Fas expression during cancer formation in BE. One study reported Fas expression in 8 of 8 esophageal adenocarcinomas, whereas another study reported an absence of Fas expression in 70% of esophageal adenocarcinomas.^{133, 134} FasL expression has been reported in both esophageal adenocarcinomas and their associated metastasis.¹³⁵

The Bcl-2 family of proteins, which includes both pro-apoptotic members (e.g. Bax) and anti-apoptotic members (e.g. Bcl-2 and Bcl-XL), are involved in BE.^{136, 137} Expression of the anti-apoptotic proteins Bcl-2 and Bcl-XL, and the pro-apoptotic protein Bax, have been found in non-dysplastic IM, low and HGD, and in adenocarcinomas.^{136–138}

Barrett's cells also use other mechanisms for avoiding autophagy and apoptosis including, inactivation of P53, downregulation of 15-lipoxygenase-1 (15-LOX-1),¹³⁹ overexpression of cyclooxygenase-2 (COX-2),^{140, 141} expression of nuclear factor kappa B (NF- κ B), and inactivation of Beclin-I.^{140–142} There are many other mechanisms that have been proposed as mediators of progression to cancer in BE. Some of these include increased telomerase expression, increased VEGFA and C, decreased membrane E cadherin and β -cadherin, increased MMP-7 and MMP-9, increased markers of EMT such as 2EB1/2EB2 and TGF-B1.^{125, 143–147} As BE's epithelial cells progress to cancer, they typically manifest aneuploidy, a marker of genomic instability. Aneuploid cells are at increased risk for neoplastic progression.¹⁴⁸ As discussed further below, genomic instability is a useful marker of progression in BE.

PREDICTORS OF PROGRESSION

Given the limitations in evaluation of dysplasia assessment by pathologists, many investigators have sought alternative, more objective, methods to assess risk of cancer progression in BE. These include the relevance of the gross (endoscopic) appearance of the dysplastic lesions, the extent of dysplasia, and a variety of IHC and molecular markers.

Endoscopic Factors

Endoscopically, dysplasia in association with visible nodules, ulcers or strictures has been shown to be associated with an increased risk of synchronous, or metachronous, adenocarcinoma. In a study by Buttar et al., 60% of patients with dysplastic nodules developed adenocarcinoma compared to only 23% of patients without nodules at endoscopy.¹⁴⁹ Thurberg et al. studied dysplastic lesions in BE that grew as exophytic, sessile or stalked polypoid lesions and found that these polypoid dysplastic lesions in BE showed a high association with HGD and adenocarcinoma within the polyp, as well as in adjacent flat mucosa.¹⁵⁰ Montgomery et al. showed that dysplasia associated with frank ulceration increased the chance of detecting adenocarcinoma at the time of esophageal resection.¹⁵¹ Although poorly studied, the presence of strictures naturally increases clinical suspicion for adenocarcinoma. In BE patients without dysplasia, the length of the BE segment and the presence of a hiatal hernia have also been shown as risk factors for progression to HGD or cancer.^{152, 153}

Extent of Dysplasia

Adenocarcinoma in BE develops within a field of clonally aberrant cells that expand to involve wide areas of mucosa.^{154–156} Several studies have shown a strong correlation between the extent of dysplasia and the risk of adenocarcinoma. In a long-term, prospective follow-up study of 77 BE patients in whom 44 patients eventually developed carcinoma, the extent of dysplasia was strongly associated with development of cancer.⁷⁵ In another study by Reid et al., the 5-year risk of cancer in patients with prevalent HGD was 59% compared with only 31% for patients with incident HGD.¹¹³ However, two other studies that evaluated the extent of dysplasia in biopsy specimen showed contrasting results, but overall suggested that the finding of diffuse HGD, characterized by dysplasia in more than 1 biopsy at different levels of the esophagus, or involving >5 crypts in 1 biopsy sample, was associated with subsequent adenocarcinoma or adenocarcinoma at the time of resection.^{149, 157} Currently, there are no clinical guidelines offered with regard to evaluation of extent of dysplasia for the purpose of stratifying patients into low and high risk groups.

Immunohistochemical and Molecular Markers

Genetic biomarkers of progression to permit selection of high-risk patients are a subject of ongoing research in BE. Many potential IHC and molecular biomarkers have been evaluated and these include many of the same ones described above for use in diagnosing dysplasia, such as DNA content abnormalities (aneuploidy/tetraploidy), inactivation of tumor suppressor p53 gene by IHC or DNA analysis, methylation markers, alterations in the synthesis of Lewis (Le) antigens and lectin proteins, among many others.^{102, 158}

p53 abnormalities have been studied as an adjunct marker of neoplastic progression and risk stratification in BE. In BE progression, p53 function is most often altered or lost by either mutation or loss of heterozygosity (LOH). Several studies have suggested that aberrant p53 expression is associated with an increased risk of neoplastic progression.^{97, 103, 104, 159–161} In a large case-control study of >12,000 biopsies from 635 BE patients, p53 overexpression was associated with an increased risk of neoplastic progression in patients with BE after adjusting for other confounding factors, such as length of BE (adjusted relative risk (RR) of 5.6), but the risk was even higher with loss of p53 expression (RR of 14.0).¹⁵⁹ In another retrospective case-control study of 16 BE patients, p53 positivity showed 85% sensitivity and 75% specificity for progression of LGD to HGD or adenocarcinoma.¹⁶⁰

Genomic instability, such as copy number alterations and LOH, are very useful markers of progression in BE. Reid et al. have shown that patients with diploid baseline biopsies show a significantly lower rate of cancer progression compared to patients with either aneuploidy or tetraploidy.¹¹³

Some studies show that a combination of biomarkers, such as DNA content and LOH of p53 and p16, are more sensitive and specific indicators of progression compared to either of these individual markers alone.^{158, 162–164} In a study by Wang and colleagues, promotor methylation of both the p16 and APC genes was associated with a significantly higher rate of progression to HGD or cancer compared to BE patients without either of these abnormalities.¹⁶⁵ In a recent European case control study of 380 patients, a panel

comprising a histologic diagnosis of LGD, abnormal DNA ploidy, and *Aspergillus oryzae* lectin expression most accurately identified BE patients who progressed to HGD or adenocarcinoma.¹⁵⁸ A retrospective double-blinded validation study of eight BE methylation biomarkers proposed a methylation biomarker-based panel to predict neoplastic progression in BE with potential clinical value in improving both the efficiency of surveillance endoscopy and early detection of dysplasia.¹⁶⁶ Despite these advances, at the present time, there are no biomarkers, or panel of biomarkers, that have been validated in large prospective cohort studies. A recent international consensus group made no recommendation regarding the routine use of molecular biomarkers in clinical practice.¹²

TREATMENT

New and increasingly sophisticated endoscopic techniques are being used for the treatment of BE patients with neoplasia. These include endoscopic mucosal resection (EMR), as well as endoscopic ablation techniques, such as laser, argon plasma coagulation, photodynamic therapy (PDT), and radiofrequency ablation (RFA). Based on the results of several recent studies, RFA has largely replaced most other forms of ablation due to its high efficacy rate and low complication rate.¹⁶⁷ Endoscopic management has been shown in multiple randomized controlled trials to effectively eliminate dysplastic and metaplastic epithelium, as well as greatly reduce cancer incidence.^{167–169}

A multimodality approach, in which a tissue acquiring technique such as EMR is followed by RFA, has revealed the best results in the treatment of HGD and intramucosal adenocarcinoma in BE. For LGD, ablation therapy has also shown an advantage over surveillance alone. For instance, a recent multicenter randomized trial of 136 patients with LGD showed that ablation therapy reduced the risk of progression to HGD and adenocarcinoma from 26.5% to 1.5% compared to surveillance alone, over a three year follow-up.¹⁶⁸ Table 3 summarizes the currently recommended approach for the management and treatment of BE with either LGD, HGD, or intramucosal adenocarcinoma, based on the gastroenterology association guidelines and recommendations from a recent large-scale international expert consensus group.^{10–12, 124}

Treatment-Related Issues for Pathologists

Endoscopic Mucosal Resection (EMR)—EMR is an endoscopic procedure designed to remove mucosa and superficial submucosal tissue. EMR serves as both a diagnostic and therapeutic procedure. By providing a larger piece of tissue than biopsies, and with good orientation, EMR specimens increase diagnostic accuracy by enabling pathologists to provide more accurate pathologic diagnostic information.^{170–172} For example, in a study by Mino-Kenudson et al., 37% of cases of BE with dysplasia diagnosed in biopsies had a change of grade when evaluated on EMR specimens. Biopsies under-reported the grade of neoplasia in 21% of cases, and over-reported the grade in 16%.¹⁷² In a recent multicenter cohort study of 138 BE patients (including 15 LGD, 87 HGD, 36 early adenocarcinoma) undergoing biopsies followed by EMR within six months, EMR evaluation resulted in a change of histologic diagnosis in approximately 30% of patients, irrespective of the presence or absence of visible lesions.¹⁷³

Overall, the role of pathologists responsible for evaluation of EMR specimens is to determine an accurate grade and type of dysplasia, and if cancer is present, to provide the type and degree of differentiation, depth of invasion, presence or absence of lymphovascular invasion, as well as the status of the lateral and deep tissue margins (with regard to dysplasia and carcinoma), all of which are factors that may have implications regarding further treatment and outcome (table 4). In patients with cancer, it is important to evaluate the depth of invasion, since this feature is significantly linked to prognosis, and helps decide whether further management, such as esophagectomy, is needed.¹⁷⁴ In addition, the rate of lymph node metastasis has been shown to correlate with the depth of invasion. An important factor to consider when evaluating depth of invasion is the presence of a duplicated (more superficially located) muscularis mucosae (dMM), which is frequently present in BE (figure 9).¹⁷⁵ The presence of a new, more superficial MM, divides BE mucosa into, essentially, four compartments: 1) inner (neo) lamina propria, 2) inner (neo) MM, 3) outer (native) LP, and 4) deep (native) MM. A study on EMRs identified extensive dMM in 38% of the specimens, moderate in 33%, and minimal in 29%.¹⁷⁶ It is important for pathologists to be aware of this phenomenon, and to be able to differentiate the two layers of muscle, in order to determine the location of the true submucosa. Several authorities have proposed staging systems for cancers that invade various levels of the mucosa.^{174, 177, 178} For instance, Vieth and Stolte proposed a staging system in which M1 indicates true LP invasion, M2 represents invasion of the superficial/duplicated layer of the MM, M3 represents invasion of the space between the two layers of MM, and M4 represents invasion of the deep/true MM.¹⁷⁷ However, a recent study didn't show any differences in the rate of LN metastases between adenocarcinomas that invaded the space between dMM, and those that invaded LP/inner MM.¹⁷⁹ Most authorities (personal communications) do not advocate utilizing the "M" system in pathology reports.

In order to maximize its diagnostic potential, it is recommended that EMR specimens be mounted cleanly on a wax block, stretched gently, and then fixed for at least 12 hours in order to produce well-oriented tissue samples. Proper inking of the lateral and deep margins should be performed. Tissue sections should be obtained at not more than 2-mm intervals in order to optimize histologic evaluation. EMR specimens removed piecemeal are difficult to evaluate pathologically, resulting in decreased diagnostic accuracy and a higher rate of reported positive margins.¹⁸⁰

Endoscopic Ablation—As mentioned above, ablation techniques are increasingly used, either as the only therapeutic modality or following an EMR, in order to destroy large areas of BE and/or associated neoplastic mucosa. There are several issues related to ablation techniques that are relevant to pathologists. These include the development of BE buried under either the neo-squamocolumnar junction, or deep to regenerated islands of squamous epithelium. In both instances, this is referred to as "buried BE". Residual foci of non-buried BE is also a complication of endoscopic ablation.

Ablation usually results in replacement of columnar epithelium with squamous (neosquamous) epithelium (NSE). Histologically, NSE appears similar to pre-ablated ("normal") squamous epithelium. It has been shown that NSE is devoid of molecular aberrations characteristic of BE, which suggests that it has no malignant potential, and

represents a successful outcome of ablation.^{181, 182} For example, in a study by Pouw, et al., 100% of pre-RFA BE patients showed abnormal Ki67, p53, and FISH assays (for chromosome 1,9, p16, and p53), but post-RFA NSE showed none of these abnormalities.¹⁸¹ In another study by Paulson and colleagues in which Post-PPI NSE and adjacent BE were evaluated for p16 and p53 abnormalities, 95% of NSE specimens showed both wild-type p16 and p53 despite the presence of mutations in 1 or both of these genes in adjacent nondysplastic BE.¹⁸²

From a clinical perspective, one of the complications is that residual Barrett's epithelium and/or dysplasia may persist underneath NSE, and thus, remain invisible to the endoscopist eye, allowing "buried BE" or "buried neoplasia" to progress to carcinoma. In fact, several cases have been reported of buried BE or buried neoplasia progressing to carcinoma.¹⁸³ The prevalence rate of buried BE or buried dysplasia is variable, and is highly dependent on the type of endoscopic therapy. For example, in a recent systematic review, the prevalence of buried BE was 14% after PDT and 0.9% after RFA.¹⁸⁴ This may be grossly underestimated, since surveillance biopsies of NSE are rather superficial, and thus, may easily miss more deeply situated foci of buried BE.¹⁸⁵ In most studies, the frequency of buried dysplasia is less than the frequency of buried BE.¹⁸⁴ Histologically, buried BE may be composed of either mucous or intestinalized glands, and are histologically similar to both pre- and post-ablation nonburied BE epithelium. Unfortunately, buried dysplasia is difficult to interpret because the features that pathologists often use to determine the grade of dysplasia, such as involvement of the full length of the crypt and the presence or absence of surface maturation, cannot be evaluated easily in buried glands covered by NSE.

The biologic potential of buried BE is also a subject of ongoing investigation. Several studies have suggested that post ablation buried BE may have less biologic potential than nonburied BE.^{186–188} For instance, in a study by Hornick et al., post-PPI-treated buried BE showed a significantly lower Ki-67 crypt proliferation rate compared to non-buried BE. However, the frequency of p53 and cyclin D1 overexpression was similar. Interestingly, in that study, buried BE not exposed to the lumen showed significantly lower crypt proliferation capability than buried BE that was exposed to the lumen.¹⁸⁷ In another study by the same group, post-PDT buried BE showed significantly lower crypt proliferation rates and significantly fewer DNA alterations, as determined by high-fidelity image cytometry on microdissected crypt cells.¹⁸⁶ In a recent study by Basavappa, et, DNA content as well as proliferative index of cells in buried BE were compared to those of normal surface BE. Although no significant differences were detected between the two with regard to DNA ploidy, buried BE cells were negative for markers of proliferation in contrast to surface BE.¹⁸⁸

Unfortunately, incomplete response, and recurrence of BE and dysplasia remain complications after complete endoscopic therapy, especially if the procedure is not performed by experienced endoscopists. Recurrence of BE after complete endoscopic therapy has been reported up to 30% at two years which emphasizes the importance of continuing long-term endoscopic surveillance.¹⁸⁹

SUMMARY

This review provides a summary of our current understanding of the biology and pathophysiology of BE and associated cancer development, with an accent on the pathologic role in evaluating patients with this disease, and the difficulty and limitations of doing so. Despite many recent advances in our understanding of the pathogenesis, molecular biology and pathology of BE and associated neoplastic lesions, there remains many ongoing controversies and challenges that need to be solved. The search for more sensitive and specific risk factors of progression, and biomarkers of cancer development, is important given the limitation of evaluating goblet cells and dysplasia in biopsy specimens and the lack of understanding of the reasons why the majority of patients do not progress to cancer. With the development of new and improved endoscopic diagnostic and treatment methods, such as radiofrequency ablation, the challenges of diagnosis and risk assessment may soon be replaced by challenges related to complications of treatment and recurrent or residual disease. A universal definition of BE is needed in order for future studies to provide meaningful and consistent data that can be applied to patients worldwide.

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Figure 1. Barrett's esophagus. The epithelium is composed of columnar epithelium with goblet cells as well as intervening non-goblet columnar cells. Paneth cells are present as well. The crypts show slight architectural irregularity and budding. The lamina propria shows a mild lymphoplasmacytic infiltrate. A few mucous glands are present in the basal portion of the mucosa (A) The glandular compartment in this case shows a mixture of mucous and oxyntic glands (B).

Figure 2.

Barrett's esophagus with pseudogoblet cells. Distinction between pseudogoblet cells (arrow) and true goblet cells (asterisk) is difficult and subject to observer variability. In contrast to the latter, pseudogoblet cells are often arranged in linear rows, and show distended cytoplasm without the characteristic triangle-shaped nucleus of true goblet cells.

Figure 3.

A) Biopsy of the squamocolumnar junctional mucosa in a patient with an irregular Z-line. Squamous epithelium is present adjacent to mucinous columnar epithelium. A mixture of mucous and oxyntic glands are present in the lamina propria. There is a mild degree of inflammation in the lamina propria. Histologically, it is not possible to determine whether the columnar mucosa in this biopsy represents metaplastic esophageal columnar epithelium or proximal gastric (cardia) mucosa. This distinction is best performed endoscopically by determining if this biopsy was obtained proximal or distal to the gastroesophageal junction. Landmarks of esophageal location, such as esophageal submucosal glands or ducts (B, arrow) or multilayered epithelium (C) are not present in this biopsy. Goblet cells are not present in these cases.

Figure 4.

Pathways of Cellular Reprogramming in Barrett's Metaplasia. Potential pathways include A) Transdifferentiation, the process in which one fully differentiated cell type (i.e. squamous) changes directly into another columnar cell, without undergoing cellular division. B) Transdifferentiation can also involve de-differentiation of squamous cells to acquire properties that it had during development. This transitional cell has morphological and molecular features that are a hybrid of both cell phenotypes (i.e. squamous and columnar cells). This transitional cell can re-differentiate into its earlier columnar cell phenotype, with further transdifferentiation into gastric and intestinal-type cells, or if the inflammation subsides, re-differentiate back into squamous cells. C) Transcommitment is the process in which immature progenitor cells are reprogrammed in order to give rise to multiple cell types that comprise gastric type and then intestinal type metaplasia. Proposed origins of progenitor cells include the esophageal squamous epithelium or submucosal gland/ducts, gastric cardia epithelium or circulating bone marrow derived cells. Relevant transcription factors that determine a columnar or intestinal phenotype are indicated in bold.

Figure 5.

Spectrum of dysplasia in BE. A) Negative. Although a mild degree of nuclear enlargement and hyperchromasia is noted, it is limited to the normal proliferative zone and there is evidence of surface maturation. B) Low grade dysplasia. The epithelium shows hyperchromatic, slightly stratified, enlarged and elongated nuclei with increased mitosis. Note the sharp transition from the non-dysplastic epithelium on the left to the dysplastic area on the right. C) High grade dysplasia. Overall, there is greater degree of cytologic and architectural atypia. The nuclei are larger in size, the N/C ratio is increased and there is significant loss of nuclear polarity, and crowded architecture. D) Intramucosal adenocarcinoma. Features may include dilated glands with intraluminal debris, fused tumor glands, and abortive glands infiltrating the lamina propria.

Figure 6. Crypt dysplasia, low grade. The epithelium shows cytologic features of low grade intestinal-type dysplasia (nuclear enlargement, hyperchromasia, and elongation, stratification, mucin depletion) limited to the basal portions of the crypts (surface maturation is present). Note the absence of active inflammation in the lamina propria.

Figure 7.

Unconventional types of dysplasia. A and B) Foveolar dysplasia, low grade. The cells contain mucin reminiscent of gastric foveolar epithelium. C) Foveolar dysplasia, high grade. There is more architectural complexity with back-to-back crypts composed of cuboidal cells with enlarged rounded nuclei and increased N/C ratio. Note the lack of nuclear stratification characteristic of conventional intestinal-type dysplasia. D) Serrated dysplasia, low grade. The epithelium has a luminal saw-tooth appearance, abundant hypereosinophilic cytoplasm, and nuclear stratification.

Figure 8.

Molecular biology of neoplastic progression in Barrett's esophagus. Some of the major genetic alterations, and the histologic stage at which each genetic change has been identified during progression to cancer are illustrated. These genetic alterations allow benign Barrett's cells to acquire core cancer hallmarks. The two enabling hallmarks of cancer are also depicted. COX-2, cyclooxygenase-2; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; APC, adenomatous polyposis coli; MMP, matrix metalloprotease.

Figure 9.

Endoscopic mucosal resection specimen showing a superficial well-differentiated adenocarcinoma. Note the presence of a duplicated muscularis mucosa (MM), consisting of a new (superficial) layer (arrows) and the original (deep) layer (asterisk). Note the presence of fragmentation and duplication of the muscularis mucosa (asterisks). In this case, adenocarcinoma has infiltrated into, and through, the superficial MM and into the “neo”-lamina propria. The original MM is also present as a fragmented layer at the bottom of the image. This cancer is still considered “intramucosal” because it has not penetrated the original MM.

Table 1

Histologic features useful to differentiate metaplastic epithelium in the distal esophagus (BE) from the normal gastric cardia in biopsies obtained from GEJ

Feature	Columnar metaplasia of esophagus	Gastric Cardia
Presence of esophageal glands and/or ducts	+	–
Multilayered epithelium	+	–
Esophagitis with features suggestive of reflux	+	+/-
H. pylori gastritis	+/-	+
Squamous epithelium overlying crypts (buried columnar epithelium)	+	–
Marked crypt distortion and budding combined with gland loss	+	–
Incomplete IM >> complete IM	+	+/-
Length of mucosa occupied by mucous or mixed mucous/oxynitic glands >0.5 mm	+	–

BE: Barrett esophagus, GEJ: Gastroesophageal junction, IM: Intestinal metaplasia, +: present, –: absent

Table 2

Histologic features of subtypes of dysplasia in BE

Type of Dysplasia		Histology	
		Architecture	Cytology
Intestinal	LGD	Relatively preserved crypt architecture	Contains goblet cells Nuclear enlargement and elongation (2–3× lymphocytes). Dense chromatin pattern Increased N/C ratio Nuclear stratification limited to basal half of cell cytoplasm Preserved or only mild loss of nuclear polarity Increased mitoses, usually limited to crypts Few, if any, atypical mitoses
	HGD	Irregular size and shape of crypts, crowded crypts, intraluminal budding or cribriforming	Nuclear enlargement (3–4× lymphocytes) Full-thickness nuclear stratification Mild to marked nuclear pleomorphism Irregular nuclear contours Prominent loss of nuclear polarity Mitoses on surface epithelium Increased number of atypical mitoses
Gastric (foveolar)	LGD	Preserved	Few or no goblet cells Mucinous cytoplasm Nuclei are uniform, small or slightly enlarged, round to oval-shaped and basally located No stratification Mitoses may be present No atypical mitoses May show reverse maturation
	HGD	Crypts are compact, elongated and show extensive branching and complexity with little intervening lamina propria	No goblet cells Mucinous cytoplasm or an absence of cytoplasmic differentiation Nuclear enlargement Increased N/C ratio Mild to moderate pleomorphism Variably prominent nucleoli No reverse maturation
Serrated	LGD	Luminal saw tooth or serrated architecture	Goblet cells may be present but are usually decreased in number Hypereosinophilic cytoplasm Nuclei are small and oval-shaped Mild nuclear stratification Mitoses in basal portion of crypts
	HGD	Luminal saw tooth or serrated architecture	Hypereosinophilic cytoplasm Nuclear enlargement Increased N/C ratio Increased nuclear stratification and pleomorphism with loss of polarity Increased mitotic rate with increased atypical mitoses

BE: Barrett's esophagus, LGD: Low grade dysplasia, HGD: High grade dysplasia

Table 3

Guideline for management of patients with BE and BE related neoplasia

Diagnosis	Management		
	AGA ¹	British Society of Gastroenterology ²	International consensus group (BOB CAT) ³
BE, no dysplasia	Endoscopic surveillance (every 3–5 years)	Symptomatic BE patients with multiple risk factors (at least three of age > 50 years, white race, male sex, obesity): endoscopic screening can be considered. (The threshold of multiple risk factors should be lowered in the presence of family history) BE patient but low-risk: surveillance is not feasible or justified.	Symptomatic BE patients that are high risk with factors including but not limited to: age > 50 years, white race, male sex, hiatal hernia, obesity or tobacco use: endoscopic surveillance (every 3–5 years) BE patient but low-risk: surveillance is not recommended.
BE, indefinite for dysplasia	Guideline not included in AGA	Repeat endoscopy in 6 months with optimization of acid suppressive therapy.	Close follow-up, with short intervals between surveillance (within 1 year), and careful biopsy sampling to detect prevalent neoplasia. Increase acid suppressive therapy
BE, LGD	Confirmed by at least one additional pathologist, preferably one who is an expert in esophageal pathology: surveillance (6–12 month) or endoscopic eradication therapy	Diagnosis needs to be confirmed by two pathologists. Endoscopic surveillance at 6 month intervals. Ablation therapy cannot be recommended routinely until more data is available.	Needs to be confirmed by at least 2 specialist GI pathologists: If low risk LGD (i.e. present on only one occasion): Continued surveillance (6–12 month). Confirmed absence of LGD after two consecutive endoscopies can revert to routine surveillance. If high risk LGD (i.e. long segment, multifocal, persistent, visible lesion): Ablative therapy with follow up. If visible lesion, EMR (+ablative therapy) + follow up
BE, HGD	Endoscopic eradication therapy with RFA, PDT, or EMR. BE, visible lesions: EMR to remove lesions In the absence of eradication therapy: surveillance every 3 months.	BE, HGD without visible lesions: Ablative therapy (RFA preferred) with follow up BE, HGD with visible lesions: EMR to remove lesions, followed by ablation (RFA preferred) to eradicate the remaining BE, regardless of the presence or absence of dysplasia	BE, HGD without visible lesions: Ablative therapy (RFA preferred) with follow up BE, HGD with visible lesions: EMR to remove lesions, followed by ablation (RFA preferred) to eradicate the remaining BE, regardless of the presence or absence of dysplasia

BE: Barrett's esophagus, LGD: Low grade dysplasia, HGD: High grade dysplasia

¹Spechler SJ, Sharma P, Souza RF, et al. American Gastroenterological Association medical position statement on the management of Barrett's esophagus. *Gastroenterology*. 2011;140:1084–1091.²Fitzgerald RC, di Pietro M, Ragunath K, et al. British Society of Gastroenterology guidelines on the diagnosis and management of Barrett's oesophagus. *Gut*. 2014;63:7–42.³Bennett C, Moayyedi P, Corley DA, et al. BOB CAT: a large-scale review and delphi consensus for management of Barrett's esophagus with no dysplasia, indefinite for, or low-grade dysplasia. *Am J Gastroenterol*. 2015.

Table 4

Role of the pathologist in evaluating endoscopic mucosal resection (EMR) specimens

DIAGNOSTIC
<ul style="list-style-type: none">• Careful gross examination of the specimen, including size, and designation of lateral and deep margins• Type of epithelium at lateral margins (squamous versus metaplastic, dysplasia or carcinoma)• Grade of lesion (LGD, HGD, intramucosal or invasive adenocarcinoma)• Degree of differentiation (if adenocarcinoma)• Depth of invasion (with careful attention to the anatomic landmarks such as duplicated muscularis mucosae)• Presence or absence of lymphovascular invasion

LGD: Low grade dysplasia, HGD: high grade dysplasia, RFA: radiofrequency ablation