Histopathology of Barrett’s esophagus after ablation and endoscopic mucosal resection therapy

This review focuses on the histopathological evaluation of endoscopic mucosal resection (EMR) specimens in Barrett’s esophagus, and on the histopathological, biological, and molecular properties of postablation Barrett’s esophagus. EMR may be used for both diagnostic and therapeutic purposes. Diagnostic accuracy regarding the grade and stage of neoplasms is improved with the use of EMR, but the value of this technique for treatment is more controversial because of the high prevalence rate of positive margins and the rate of metachronous lesions found elsewhere in the esophagus during follow-up. Ablation techniques, such as argon plasma coagulation, photodynamic therapy, and radiofrequency ablation, are used increasingly for the treatment of Barrett’s esophagus and related neoplasms, often in combination with EMR. A common problem after use of these techniques is the development of islands of neosquamous epithelium (NSE) which can overlie buried Barrett’s (and/or dysplasia) epithelium. This is, therefore, concealed to the endoscopist’s view and may be allowed to progress to cancer without detection. NSE is histologically similar to normal esophageal squamous epithelium and does not possess the molecular aberrations characteristic of Barrett’s esophagus. In contrast, residual nonburied Barrett’s esophagus shows persistent pathologic and molecular abnormalities and may progress to cancer upon long term follow-up. The biological potential and rate of progression of nonburied residual Barrett’s esophagus following ablation is unclear, but some preliminary studies suggest that the risk may decrease. Buried nondysplastic Barrett’s esophagus appears to show decreased biological potential and this may be related to protection from the contents of the lumen by the barrier function of the overlying NSE. On the other hand, anecdotal reports have suggested that buried dysplasia may progress to cancer in some instances.

Brief Overview of Histopathology of Barrett’s Esophagus

Nondysplastic epithelium

The American College of Gastroenterology (ACG) defines Barrett’s esophagus as endoscopically recognizable columnar metaplasia of the esophagus, which is confirmed to have intestinal metaplasia (i.e. goblet cells) in mucosal biopsy specimens [1]. By definition, this does not include patients who have intestinal metaplasia of the gastric cardia, in whom the risk of neoplastic progression is considered extremely low. However, the definition of Barrett’s esophagus varies worldwide. For instance, the British Society of Gastroenterology does not require goblet cells to be identified in biopsy specimens in order to diagnose Barrett’s esophagus [2]. This is based on recent data that suggests that metaplastic non-goblet columnar epithelium in the esophagus is potentially at risk for neoplastic change [3,4], and that the chances of detecting goblet cells are related to many factors, such as sampling error, the length of Barrett’s esophagus, the age of the patient and the location of biopsies [5–8]. Nevertheless, Barrett’s esophagus is characterized by both epithelial and mesenchymal changes in the esophagus [9,10]. Epithelial changes include the formation of surface mucinous columnar cells, either with or without goblet cells, enterocytes, and cells with combined intestinal and gastric features [9]. Acute and chronic inflammation and even ulceration may be present and is common in patients with persistent gastroesophageal reflux disease (GERD). The underlying glands are typically composed of either pure mucous cells, pure oxyntic cells, or more often, a mixture of both types of cells. Mucous glands predominate in most individuals with Barrett’s esophagus, particularly in the more
proximal regions of the columnar segment, whereas oxyntic-type glands are more common in the distal segment. Goblet cells are detected more often in the proximal region of Barrett’s esophagus, near the neosquamocolumnar junction [8]. Intermediate cell types, such as pseudogoblet cells, ciliated mucous cells, and multilayered epithelium, are common [9,11]. Multilayered epithelium reveals cells with squamous and columnar differentiation, and is thought to represent an intermediate or transitional stage in the conversion of squamous to columnar epithelium in the esophagus [11]. Characteristic mesenchymal changes include duplication of the muscularis mucosa, in which the newly developed (more superficial layer) of muscularis mucosa is in contact with the crypt bases, and is often frayed and stranded in appearance [10] (Fig. 1).

A thin, often edematous lamina propria usually separates the more luminally situated muscularis mucosa from the deep (original) muscularis mucosa, which, in fact, represents an extension of the original muscularis mucosa of the squamous-lined esophagus. The presence of a double muscularis in Barrett’s esophagus makes interpretation of dysplasia and adenocarcinoma difficult, particularly in biopsy specimens (see the section below on endoscopic mucosal resection [EMR] specimens). Determination of the precise location of neoplastic involvement in Barrett’s esophagus is important since several studies have shown that tumors that infiltrate into, and even through, the new muscularis mucosa, have a very low risk of metastasis (<1%) compared with tumors that penetrate the submucosal layer (<44%) [12].

**Dysplastic epithelium**

Dysplasia in Barrett’s esophagus is classified as negative, indefinite, or positive (either low or high grade) according to a classification system developed initially for dysplasia in inflammatory bowel disease [13]. Some pathologists, particularly in Europe and Asia, prefer the Vienna classification system, which uses the term “intraepithelial neoplasia” instead of “dysplasia.” In this classification, “high grade dysplasia,” “carcinoma in situ,” and “suspicious for invasive carcinoma” are all grouped into one category of “high grade intraepithelial neoplasia” [14]. Non-dysplastic Barrett’s esophagus (“negative for dysplasia”) represents the normal background columnar epithelium with regenerative changes. Low and high grade dysplasia are generally distinguished from regenerating epithelium on the basis of a combination of cytologic and architectural features [15]. By convention, low grade dysplasia shows atypical epithelium characterized by the presence of stratified cells with hyperchromatic, elongated nuclei, clumped chromatin, irregular contours, increased mitoses and slight loss of cell polarity, but with preservation of crypt architecture. With progression to high grade dysplasia, the extent of cytopathologic and/or architectural complexity increases. Findings include increased crypt complexity, crowding, irregularity, and branching, and cytologically there is more pronounced nuclear stratification, loss of cell polarity, pleomorphism, and atypical mitotic activity (Fig. 2).

It is important to note that dysplasia develops on a continuous (linear) scale, and, thus, it is often difficult to distinguish the high end of low grade dysplasia from the low end of high grade dysplasia [15,16] (Fig. 3).

In fact, recently, strong morphologic, immunohistochemical, and molecular evidence suggests that dysplasia begins initially in the crypt bases and progresses with time to involve the full length of the crypts and surface epithelium [17]. When cells breach the basement membrane and infiltrate into the lamina propria or submucosa, the neoplastic condition is considered to be “adenocarcinoma.” Intramucosal adenocarcinoma is defined as tumors that infiltrate into, but not through, the deep (original) muscularis mucosa. Tumors that penetrate through the deep muscularis mucosa are considered submucosally invasive adenocarcinomas. The problems related to interpretation of dysplasia and the depth of adenocarcinoma are highlighted in the section below on the pathologic evaluation of EMR specimens.
Due to overlap between regenerating and dysplastic epithelium, and the often subtle gradation of changes that occur with progression of dysplasia in Barrett’s esophagus, there is a significant degree of interobserver variability in the diagnosis of dysplasia [13,16]. The highest degree of variability occurs at the low and high ends of the spectrum (i.e. separating regeneration from low grade dysplasia and separating high grade dysplasia from adenocarcinoma). As a result, the ACG has strongly recommended that all potential dysplasia diagnoses be confirmed by at least one experienced gastrointestinal pathologist before initiation of patient management [1]. This point is further emphasized by the results of several recent studies that show a strong correlation between the number of pathologists who agree with a dysplasia diagnosis, and the rate of neoplastic progression [18,19]. Unfortunately, in the general pathology community there is a tendency to overdiagnose high grade dysplasia [20]. For instance, in one study, nearly 40% of patients diagnosed with high grade dysplasia by a general pathologist had their diagnosis amended to a lower grade of dysplasia by three experienced gastrointestinal pathologists [20]. This is even more important for the evaluation of buried dysplasia, whether it occurs following proton pump inhibitor (PPI) treatment or ablation therapy, since the residual effects of inflammation and healing, combined with the lack of surface epithelium and proper crypt orientation, makes diagnosing buried dysplasia even more difficult.

Cases may be considered indefinite for dysplasia for a variety of reasons, such as when atypical changes are borderline in appearance between regeneration and true dysplasia, or when technical issues, such as tangential or thick tissue sectioning, poor orientation, or lack of surface epithelium makes interpretation difficult [15].

Due to overlap between regenerating and dysplastic epithelium, and the often subtle gradation of changes that occur with progression of dysplasia in Barrett’s esophagus, there is a significant degree of interobserver variability in interpretation. The basal portions of the crypts show low grade features characterized by nuclei limited to the basal half of the cell cytoplasm and without loss of polarity. However, the upper portions of one crypt, and the surface epithelium, show enlarged nuclei and an increased degree of nuclear stratification, focally involving the full thickness of the cell cytoplasm, and loss of cell polarity. The architecture of the lesion is well maintained.

Histopathology of Endoscopic Mucosal Resection (EMR) Specimens

In contrast to other types of ablative therapy (e.g. laser and photodynamic therapy [PDT]) that destroy neoplastic tissue, EMR removes mucosal and often superficial submucosal tissue, which allows for more accurate histologic evaluation and grading of dysplasia, and evaluation and confirmation of depth (stage) of invasion in cases where adenocarcinoma is present [21]. EMR is also used therapeutically to remove high grade dysplasia and intramucosal carcinoma, but the use of EMR for this purpose is more controversial. Multiple techniques of endoscopic resection have been developed. However, the superiority of one technique versus another has not been fully established with regard to the use of EMR for either diagnosis or therapy.

Pathologic evaluation of EMR specimens

Evaluation of tissue margins and depth of invasion are important when the histopathologist is confronted with an EMR specimen. EMR specimens should be mounted on a wax block and stretched gently prior to fixation for at least 12 hours. The specimen should also be photographed, a practice that can help correlate the histologic with the endoscopic findings. The deep and circumferential margins should be marked with ink in order to evaluate the margins of resection appropriately. Tissue sections should be obtained from fixed EMR specimens at 2-mm intervals, which optimizes evaluation of the grade and stage of the neoplasm, the presence or absence of vascular involvement, and the status of the lateral and deep margins. If the specimen is wide enough, radial sections should be submitted as well. For small EMR specimens, both ends of the specimen should be submitted en face. EMR specimens removed piecemeal are difficult to stage accurately. In some series, up to 26% of EMR specimens are fragmented [22], a problem that may become more common with the increasing use of the band ligation technique.

Microscopic evaluation of EMR specimens should always include an assessment of the status of the margins and the grade and stage of the neoplasm (low or high grade dysplasia, intramucosal or submucosal carcinoma). The presence or absence of vascular invasion should also be noted for cases with an invasive tumor. In one series of 27 EMR specimens, microscopic evaluation was considered complete in only 4% of cases [22]. In that study, the circumferential margin was positive in 68%, and both the deep and circumferential margins were positive in 28% of cases [22].

The status of the tissue margins is the most important prognostic parameter in EMR specimens. For instance, when adenocarcinoma is noted at a surgical margin, the risk of recurrence is 37% – 50% [23 – 25]. In one series of gastric EMR specimens, the rate of tumor recurrence in cases that revealed a positive circumferential margin was 33% to 50% when one or more tissue margins were involved, respectively [23]. Technical artifacts, such as hemorrhage and electrodialthermic burns, may be present in EMR specimens and may limit histologic interpretation [23].

Diagnostic value of EMR specimens

EMR specimens enhance the ability of pathologists to establish accurate diagnoses and stage tumors, compared with mucosal biopsies [22 – 24]. In one study by Mino-Kenudson et al. in 2005, a change in the original mucosal biopsy diagnosis was made in...
37% of cases after evaluation of the patient’s EMR specimen [22]. In fact, in that study, biopsies underreported the grade of neoplasia in 21% of cases, and overreported the grade in 16%. Similar results were reported by Peters et al. with post-EMR diagnoses differing from pre-EMR diagnoses in 49% of 150 cases. In that study, 62% of cases in which a change of diagnosis was made led to a clinically relevant change in treatment policy [26]. Finally, in a series of 48 patients, Lightdale and colleagues reported that on the basis of EMR specimen evaluation, six of 25 patients (24%) with a diagnosis of high grade dysplasia in biopsy specimens had their diagnosis staged up to intramucosal adenocarcinoma, and six of 15 patients (40%) with a biopsy diagnosis of intramucosal adenocarcinoma had the diagnosis staged up to submucosal invasive carcinoma [25]. Discrepancies between biopsy and EMR specimens were shown to be more prevalent when lesions were larger in size (> 10 mm) and when less extensive biopsy sampling was performed prior to EMR [27].

As noted above, interobserver variation is a major problem in the pathologic evaluation of Barrett’s esophagus-associated neoplasms. However, using EMR specimens, the degree of interobserver agreement between pathologists for Barrett’s esophagus-related neoplasms is significantly higher compared with evaluation of biopsy specimens [28]. Improvement in diagnostic agreement is likely related to the larger size of tissue sampled in EMR specimens compared with biopsies, and the enhanced ability to evaluate important mucosal landmarks, such as the duplicated muscularis mucosa.

EMR has also been shown to add value to preoperative staging by endoscopic ultrasound (EUS). With the development of endoscopic therapy, pathologic discrimination of intramucosal adenocarcinoma from submucosal adenocarcinoma is critical. For instance, intramucosal tumors may be treated endoscopically by gastroenterologists, whereas submucosal cancers often require surgical resection. EUS has shown a 72%–95% accuracy rate in distinguishing mucosal from submucosal carcinoma [21]. In fact, with EUS, overstaging of neoplasms occurs in up to 12.5% of cases, and understaging occurs in 16% to 20% [21]. In a series of patients in whom EMR specimens revealed submucosal invasion in those originally staged at EUS as having intramucosal adenocarcinoma, Larghi et al. showed that submucosal invasion was highly associated with the appearance of nodules at endoscopy [26]. In fact, submucosal invasion was uncommon in endoscopically “flat” lesions. In these cases, histologic evaluation of EMR specimens offered added value over staging by endoscopic ultrasound. Recently, in a study of 36 EMR specimens, Mandal et al. confirmed that common causes for overstaging by endoscopic ultrasound were large vertical dimension of the tumor and the presence of a duplicated muscularis mucosa [29].

**Therapeutic value of EMR**

The use of EMR as an alternative to esophagectomy for the treatment of dysplasia/superficial carcinoma in Barrett’s esophagus is currently being investigated. For instance, one European prospective series of 100 patients with “low risk” adenocarcinomas (well to moderately differentiated, ≤20 mm in diameter, and confined to the mucosa without angiolymphatic invasion or ulcer on endoscopy) reported an overall recurrence rate of only 11%, and a mortality rate of 0%, after a mean follow-up of 36.7 months [30]. Unfortunately, the usefulness of EMR as a therapeutic procedure may be limited by high reported rates of positive margins, which ranges from 62% to 83% in some studies, and by the occurrence of metachronous lesions elsewhere in

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**Fig. 4** a Biopsy of a patient with Barrett’s esophagus prior to endoscopic mucosal resection (EMR). The biopsy shows high grade dysplasia characterized by epithelium with marked cytologic atypia and architectural distortion. However, in this biopsy, there is no definite evidence of adenocarcinoma. b EMR of the area biopsied in a Evaluation of the entire endoscopic mucosal resection shows areas containing more severe degrees of epithelial atypia, and definite areas of intramucosal adenocarcinoma (center of field). However, the lesion does not infiltrate into the muscularis mucosa. c High power view of a portion of the mucosa illustrated in the EMR specimen of b Complex glands containing marked cytologic atypia and necrosis are characteristic features of intramucosal adenocarcinoma.
Barrett’s esophagus during follow-up [22, 31] Tumor grade has been reported to be important in the ability to obtain negative tissue margins. Unfortunately, some studies did not evaluate resection margins systematically [21,32]. Some studies also suggest that the anatomic site of positive margins is important with respect to recurrence. For instance, 44% of EMR specimens with ≥1 positive lateral/circumferential margin and a negative deep margin showed no evidence of residual tumor and/or recurrence after a median follow-up of 23 months in one study [22]. However, 86% of EMR specimens with a positive deep margin showed residual tumor despite use of PDT in some of the cases. These data were also recently confirmed by Prasad et al., who reported, in a study involving 25 patients, that none of those with negative mucosal margins showed residual tumor, whereas 50% of patients with submucosal carcinoma showed residual tumor at the EMR site, at the time of esophagectomy [33]. Furthermore, 30% of patients with submucosal carcinoma revealed lymph node metastasis. This again, strongly supports the need for additional therapy in patients with positive vertical margins, whereas for patients with positive lateral resection margins endoscopic follow-up or additional endoscopic ablation therapy may be justified. Fortunately, the frequency of complications associated with EMR is modest. Significant bleeding is observed in up to 14% of cases. However, most patients with bleeding are managed endoscopically in an outpatient setting. Perforation is observed in 1.8% of procedures, and may even be treated effectively by medical therapy in some circumstances. There have been no reports of deaths due to EMR [21,30,34].

HISTOPATHOLOGY OF POSTABLATION BARRETT’S ESOPHAGUS

Neosquamous epithelium
The replacement of Barrett’s esophagus (“re-epithelialization”) by newly developed squamous epithelium, referred to as neosquamous epithelium (NSE) [35], occurs with all forms of ablation, such as laser, argon plasma coagulation, PDT, and radiofrequency ablation (RFA), as well as with PDT treatment. The prevalence of NSE has been shown to vary depending on the type of therapy, but its development is a common phenomenon even in patients treated with PPIs without ablation [36]. In some studies, up to 77% of Barrett’s esophagus patients have shown macroscopic or microscopic evidence of NSE following treatment with PPIs [37,38]. In fact, small islands of NSE may be missed endoscopically, being detected only by microscopic examination of biopsy tissue [36]. In one study of 44 Barrett’s esophagus patients with NSE, only 43% of cases were detected endoscopically [36]. The frequency of development of NSE following ablation is higher, and in fact, reaches 100% in patients treated with PDT [37–39]. In RFA-treated patients, virtually all develop complete replacement of the Barrett’s esophagus segment with NSE [40–43].

Pathologic and molecular features of NSE
The extent of development of NSE also varies according to the type of therapy [37,39,44–48]. The degree of NSE may vary from isolated islands at one end of the spectrum to complete replacement of the Barrett’s esophagus segment at the other [49]. The pathologic features of NSE have not been systematically investigated. However, anecdotal experience suggests that NSE is similar to normal esophageal squamous epithelium, being composed of 1–2 layers of basal cells, overlying suprabasal cells, and multiple layers of progressively more mature squamous cells. NSE often shows regenerative changes, such as basal cell hyperplasia and elongation of the lamina propria papillae, but these features are often most pronounced in the early postablation period, and are considered morphologically nonspecific.

Several studies have evaluated the molecular properties of NSE [50–52]. In the great majority of cases, NSE shows complete absence of molecular abnormalities that are typically present in adjacent, and occasionally underlying, Barrett’s esophagus. For instance, in one preliminary study by Finkelstein et al., microdissection of NSE from patients treated with Barrett’s esophagus showed an absence of mutations in genes that were present in Barrett’s esophagus columnar cells in pretreatment biopsies [52]. In another study by Paulson et al., isolated islands of NSE and surrounding Barrett’s esophagus from 20 patients were microdissected and evaluated for genetic alterations at exon 2 of p16 or exon 5–9 of the p53 gene [50]. In that study, 19 of 20 patients (95%) showed wild-type p16 and/or p53 gene expression in NSE, even though one or both of these mutations were present in surrounding Barrett’s esophagus in all cases. Similarly, Leedham et al. showed that all but one foci of NSE showed wild-type p16 and p53 point mutations after genetic analysis of laser-captured microdissected tissue from Barrett’s esophagus patients [51]. Ultimately, from a clinical perspective, there is no pathologic or molecular evidence to suggest that NSE is at risk for neoplastic progression. However, as outlined below, NSE often shows evidence of buried Barrett’s esophagus, and even buried dysplasia/carcinoma, which necessitates continued surveillance of areas of NSE in Barrett’s esophagus patients following ablation.

Origin of NSE
The location and type of the progenitor “stem” cell of NSE are essentially unknown. In fact, theories regarding the cell of origin of NSE are similar to those proposed for Barrett’s esophagus [9,11,35,39,50,51,53–56]. These include the possibility that stem cells reside in the basal layer of normal squamous epithelium at the neosquamous columnar junction, within underlying Barrett’s esophagus columnar epithelium, and/or within the esophageal glands or ducts. Recently, in vitro and experimental evidence has supported the possibility that pluripotent stem cells may be derived from either undifferentiated mesenchymal cells in the lamina propria or the bone marrow [54,55]. Several animal studies have provided strong evidence that the cell of origin of Barrett’s esophagus resides in the esophagus rather than the proximal stomach, so it is assumed that this also pertains to the cell of origin of NSE [56]. Nevertheless, some studies have shown that NSE may actually develop from several different potential sources. For instance, in one pathologic study of 10 Barrett’s esophagus patients treated with PPI at high dosage, NSE appeared to develop via spread from contiguous squamous borders, and also from the underlying glandular/ductal tissue [35]. Pathologically, NSE is often seen to involve the superficial aspects of the esophageal gland duct epithelium, which supports the possibility of stem cells in this anatomic structure [39]. In the study by Leedham et al. mentioned above, an island of NSE was seen to arise directly from an esophageal gland duct within a field of Barrett’s esophagus [51]. Certainly, the finding of wild-
studies are needed to determine the cell of origin of Barrett’s esophagus and NSE. Instances, a progenitor cell capable of differentiating into both columnar or squamous epithelium may occur. Clearly, further studies are needed to determine the cell of origin of Barrett’s esophagus and NSE.

**Residual and buried Barrett’s epithelium (“buried Barrett’s”)**

**Buried Barrett’s esophagus**

The frequency of buried Barrett’s esophagus and buried dysplasia is variable, and somewhat dependent on the type of ablation technique used [57]. For instance, earlier studies reported post-PDT rates of buried Barrett’s esophagus in the range 0%–6%, and buried dysplasia/carcinoma in 0%–3% [44]. However, in one detailed and systematic pathologic study of 33 patients with high grade dysplasia or intramucosal adenocarcinoma treated with PDT, evaluated using an extensive four-quadrant sampling protocol, buried Barrett’s was detected in 27.3% of patients prior to PDT (presumably due to PPI therapy), but this increased to 51.1% following PDT [37]. Similarly, buried dysplasia or carcinoma was noted in 12.1% of patients before PDT, and this value increased to 27.3% after PDT. Of the patients, 17 (52%) showed complete eradication of dysplasia/carcinoma, but persistence of dysplasia/carcinoma was noted in the remainder (48%). In contrast, several studies have shown a complete absence of buried Barrett’s or buried dysplasia in nearly 100% of patients after RFA [40–44]. Overall, those data include evaluation of over 5000 esophageal biopsies.

Pathologically, buried Barrett’s esophagus shows similar features to both pretreatment, and postablination, residual nonburied Barrett’s esophagus. Similar to pretreatment Barrett’s esophagus, buried Barrett’s may be composed of glands either with or without goblet cells. The glands may be mucous or oxyntic, or contain a mixture of both types of secretory cells (Fig. 5).

In the only study published to date that evaluated types of epithelium in buried Barrett’s, Hornick et al. showed that 89% of foci were intestinal in type (i.e. contained goblet cells), 11% were cardia-type (composed of mucous glands without goblet cells), and none were composed of pure oxyntic glands [36]. However, this study included only PPI-treated patients; none had received ablative therapy. Interestingly, by performing detailed and exhaustive tissue sectioning, Hornick et al. also showed that buried Barrett’s esophagus (even foci without goblet cells) showed a connection to the mucosal surface in 68% of foci, either by penetration directly through islands of NSE, or by wrapping around them. In fact, 21% of biopsies in this study showed buried Barrett’s esophagus adjacent to submucosal glands or ducts, which also supports the possibility that these latter structures contain the cell of origin of NSE.

The histopathologic features of residual nonburied Barrett’s esophagus are similar to those of pretreatment epithelium, although a detailed comparison of these two types of epithelium has never been performed. Both residual nonburied and buried Barrett’s esophagus may show marked regenerative changes, such as nuclear hyperchromaticity, nuclear stratification, prominent nucleoli, and increased mitoses, and a mild degree of acute and chronic inflammation, particularly in the first few days to weeks following ablation therapy. After a few months, lamina propria fibrosis and duplication and splitting of the muscularis mucosa may develop as well.

**Buried dysplasia**

Buried dysplasia is a common and important clinical problem in patients treated with any type of ablation therapy, perhaps with the exception of RFA (Fig. 6).

Success rates for complete eradication of residual nonburied and buried dysplasia ranges from 48% to 100% [37,44,47]. However, most studies reveal that a significant proportion of Barrett’s esophagus cases show downgrading of their neoplastic epithelium following ablation, particularly after PDT and RFA. Differences in the results of these various studies may be due to multiple reasons, such as differences in methodology, variation in sampling protocol, differences in pretreatment extent of Barrett’s esophagus, and lack of detailed histologic analysis. From a clini-
cal point of view, some patients may show eradication or downgrading of their dysplasia, post ablation, but then develop further progression with time, so continued surveillance is considered essential [58].

Pathologically, evaluation of buried dysplasia, and even buried cancer, may be quite difficult. For instance, one of the cardinal morphologic features of traditional dysplasia is involvement of the upper crypts and surface epithelium, which helps distinguish regenerating epithelium (in which surface maturation is usually present) from true dysplasia (in which surface maturation is not). Thus, evaluation of the presence or absence, and grade of dysplasia located underneath islands of NSE is challenging, due to the pathologist’s inability to evaluate the overlying surface epithelium, and to identify other architectural aberrations that help distinguish low grade from high grade dysplasia. Thus, pathologists should be extremely careful not to overdiagnose dysplasia in cases of buried “atypical” epithelium that shows borderline features between regeneration and dysplasia.

Biological properties of buried Barrett’s esophagus

Of major clinical concern is the natural history and risk of malignancy of residual nonburied and buried Barrett’s esophagus following ablative therapy. In fact, the development of occult adenocarcinoma from residual Barrett’s esophagus located underneath NSE has been reported [38,46,59]. This has raised concern that buried Barrett’s esophagus and/or buried dysplasia may go undetected endoscopically and be allowed to progress to cancer. Unfortunately, only a few studies have evaluated the biological properties of residual Barrett’s esophagus following ablation [36,60]. For instance, in a study by Hornick et al. of 12 Barrett’s esophagus patients treated with PDT, biopsies taken before PDT showed a significantly higher crypt proliferation rate and higher DNA content abnormalities (measured by image cytometry) compared with post-PDT residual Barrett’s esophagus [60]. In fact, post-PDT buried Barrett’s esophagus showed the lowest proliferation rates and a normal DNA content profile. In another study by the same group, of 44 Barrett’s esophagus patients treated with PPIs, similar results were seen [36]. Furthermore, in the latter study, buried Barrett’s esophagus glands that did not reveal a connection to the luminal surface showed the lowest crypt proliferation rates, suggesting that in buried crypts the decreased exposure to luminal contents may lead to protection, and a decreased risk of malignant progression. Alternatively however, decreased or absent sloughing of surface epithelium due to the overlying NSE barrier may lead to accumulation of genetic mutations, and expansion of abnormal clones of cells, with a propensity to progress to cancer in concealed Barrett’s esophagus.

Analysis of genetic abnormalities in residual nonburied Barrett’s esophagus and dysplasia following PDT, has been more extensively studied, and has, indeed, revealed persistent proliferative and molecular abnormalities, which suggests that this epithelium continues to be at risk of malignant progression [58,60–63]. For instance, Hage et al. evaluated loss of heterozygosity (LOH) of 8q, 9p, 17p and 18q in both pre-PDT and post-PDT Barrett’s esophagus (nonburied) and showed persistence of LOH abnormalities, following PDT, in both nondysplastic and dysplastic epithelium [62]. In another study by the same group, ki67 and p53 immunohistochemistry, and in situ hybridization for abnormal chromosome 1, were evaluated in both pre- and post-PDT biopsies of nonburied Barrett’s esophagus [63]. Persistent increased crypt proliferation was noted in post-PDT biopsies, but p53 overexpression and abnormal chromosome 1 persisted only in residual dysplastic epithelium. Finally, in a follow-up study by Krishnadath et al. in 2000, despite initial pathologic downstaging following PDT in three Barrett’s esophagus patients with high grade dysplasia, increased cell proliferation and aneuploidy re-developed in all three patients upon follow-up, despite the fact that in the initial post-PDT period, two patients showed conversion to diploidy and decreased cell proliferation [58]. Thus, it appears that even with morphologic downgrading of dysplasia following PDT, residual post-ablation nonburied Barrett’s esophagus continues to be at risk of neoplastic progression. Clearly, long term follow-up studies are needed to determine the malignancy risk of both buried and residual nonburied Barrett’s esophagus after ablation therapy.

Competing interests: None

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