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Progression of esophageal dysplasia to cancer

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The following, from the 12th OESO World Conference: Cancers of the Esophagus, includes commentaries on the evolution of low-grade squamous and glandular dysplasia to invasive carcinoma; the mutational spectra of Barrett's esophagus and adenocarcinoma; the risk of p53-immunoreactive glandular dysplasia compared to non-immunoreactive mucosa for progression to cancer; the role of lectins in progression to adenocarcinoma; and the role of racemase immunoreactivity in the prediction of risk of adenocarcinoma.

Keywords: Barrett's esophagus; esophageal adenocarcinoma; p52; racemase; lectin; OESO

Concise summary

Intraepithelial neoplasia is subclassified into two important categories: low-grade dysplasia (LGD), including mild and moderate dysplasia; and high-grade dysplasia (HGD), comprising severe dysplasia and squamous cell carcinoma *in situ*. Important studies have shown that squamous dysplasia and carcinoma *in situ* were the only histological lesions associated with a significantly increased risk of developing esophageal squamous cell carcinoma (ESCC) and that increasing grades of dysplasia were strongly associated with increasing risk, indicating that the histological grading was clinically meaningful. Recent advances in molecular biology emphasize the strict sequence LGD–intraepithelial neoplasia–HGD–intraepithelial neoplasia in the development of invasive cancer, depending on genetic and epigenetic changes that parallel the histological modifications observed in the neoplastic progression. In resection specimens, the overall frequency of loss of heterozygosity (LOH) at microsatellite loci was significantly increased as the pathological status changed from LGD to HGD and ESCC, indicating that tumorigenesis of the esophageal squamous epithelia is a progressive process involving a series of molecular alterations.

In the context of Barrett's esophagus (BE), glandular dysplasia is classified into two important categories, namely LGD and HGD, on the basis of accurate histological features and an accurate diagnostic interpretation from the pathologist. The existence of the sequence LGD–HGD–invasive cancer is supported by immunohistochemistry (IHC) and molecular biology. In particular, the application of immunohistochemical techniques revealed the existence of two pathways in the development of dysplasia in the context of BE: on one side, dysplastic cells show positivity for p16 and negativity for p53 and HER2; on the other side, dysplastic cells express p53 and HER2 and lack of p16 expression.

Esophageal adenocarcinoma (EAC) is primarily associated with obesity, gastroesophageal reflux disease (GERD), and BE. In GERD patients, the low pH, as well as the bile salts, induces expression of cyclooxygenase-2 (COX-2), which catalyzes the conversion of arachidonic acid into various prostaglandins including prostaglandin E_2 (PGE_2) and induces proliferation of Barrett's cells, with a high probability of these cells accumulating replication errors. PGE_2 also inhibits tumor surveillance by inhibiting natural killer cell activity. COX-2 may also induce the production of reactive oxygen species

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(ROS), resulting in oxidative stress and subsequent oxidative DNA damage that could contribute to carcinogenesis. The presence of genetic mutations is another important factor leading to EAC. Changes in the cell cycle of the affected epithelial cells, such as an increased number of cells in S phase, corresponding to DNA synthesis, and G_2 phase have been described in BE patients. Genomic instability in BE is also represented by widespread LOH, point mutations, alterations in microsatellite alleles, and epigenetic changes including hypermethylation of promoter regions in genes. Several data suggest that interactions between risk factors may be more important than the individual risk factors themselves in the development of EAC.

In Barrett's mucosa, is p53-immunoreactive LGD at higher risk than non-immunoreactive LGD for progressing to EAC? In order to answer this question, we have to answer two others. First, is the histologic diagnosis of LGD reproducible? Second, if it is reproducible, then is positive p53 staining of the dysplastic epithelium a better predictor of progression to carcinoma than negative staining? Several studies of dysplasia have found that reproducibility among even highly experienced pathologists is best at the highest end of the dysplastic spectrum, namely HGD and carcinoma, while it is poorest at the LGD end. Since there is no consistent definition of LGD, it is to be expected that the definitions in the literature and in the textbooks of gastrointestinal pathology will not be the same. p53 is a tumor suppressor gene, a transcription factor important in cell cycle regulation. The encoded protein is nuclear, so the immunostain is easy to interpret. Several studies have shown that p53 positivity helped in the diagnosis of LGD, and it seems that patients with p53-immunoreactive LGD are at higher risk for progression than those with p53–non-immunoreactive LGD, although this is clouded by the lack of reproducibility for the LGD diagnosis. There is recent mention of p53 immunostaining being considered as an adjunct to routine histopathologic diagnosis, hoping to improve the diagnosis of dysplasia in Barrett's mucosa.

An evolving understanding of the complex glycoprotein milieu in the normal and diseased esophagus paints an intriguing picture that suggests a re-emergent role for lectins, proteins with high sensitivity, and variable specificity for selected glycosyl moieties, in the diagnosis and management of BE and EAC. Applying lectin-affinity chromatography to serum samples using the fucosyl-specific lectins *Aleuria aurantia* lectin and *Lotus tetragonolobus* agglutinin, some studies identified a subset of proteoglycans including fetuin B, a cystatin that is somewhat overexpressed in Barrett's HGD relative to disease-free esophagus, but significantly increased in carcinoma. The relative depletion of fucosyl residues in the BE—EAC sequence is immediately applicable to routinely processed biopsy samples. Studies using a fucosyl-specific lectin derived from *Aspergillus oryzae* confirmed the expectation that decreased histochemical staining may predict the transition from BE to EAC. Further findings have led to the formulation of a robust risk-stratification biomarker panel for progression from BE to EAC. Among other lectins, the *N*-acetyl-D-galactosaminyl–selective agglutinin from *Helix pomatia* selectively labels glycans that, when present or increased in number, may be associated with progression from locally invasive esophageal disease to metastasis. Selective lectin labeling of esophageal mucosa in BE patients may prove to be a useful guide for traditional biopsy techniques. As an adjunct to confocal laser endomicroscopy, lectin labeling could reasonably be expected to increase the efficiency of both scanning and targeting of suspicious lesions.

α-Methylacyl-CoA-racemase (AMACR) is present in many dysplasias and carcinomas, Dysplastic mucosa may be $AMACR^+$ with IHC, and the question was raised whether AMACR could be of value in identifying patients at highest risk for BE. Cohort studies showed that, although mild AMACR expression was associated with a trend towards an increased risk of neoplastic progression, the risk was especially elevated with strong AMACR expression. After adjusting for histological diagnosis, only strong AMACR expression remained associated with a significantly increased risk of neoplastic progression. AMCR expression has a degree of predictability, but is not sensitive, non-dysplastic biopsies having mild and yet strong immunoreactivity.

Table 1. The subclassification of squamous intraepithelial neoplasia in the 2010 WHO edition

Squamous intraepithelial neoplasia (IEN)			
Low-grade IEN		High-grade IEN	
Mild dysplasia	Moderate dysplasia	Severe dysplasia	Squamous cell carcinoma *in situ* (CIS)

1. Can low-grade squamous/ intraepithelial dysplasia give rise directly to invasive cancer?

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The development of dysplasia in the squamous esophageal epithelium has been extensively investigated by several authors in the literature, emphasizing the role of alcohol consumption, smoking, and diet (in particular the ingestion of *N*-nitroso compounds) as main risk factors for the onset of dysplastic morphological changes.[1] In the latest WHO classification edition (2010), the term *intraepithelial neoplasia* (IEN) was introduced and subclassified into two important categories: low-grade IEN (LG-IEN), including mild and moderate dysplasia, and high-grade IEN (HG-IEN), comprising severe dysplasia and squamous cell carcinoma *in situ* (CIS; Table 1).[2] Histologically, squamous IEN is defined by both architectural and cytological abnormalities that vary in extent and severity (Fig. 1). These include loss of normal cell polarity, overlapping nuclei, and lack of surface maturation. Characteristic cytologic features include enlarged, hyperchromatic nuclei with increased nuclear-to-cytoplasmic ratio and increased mitotic activity. At low magnification, a sharp demarcation commonly delineates the neoplastic epithelium from the surrounding epithelium. The diagnostic changes characteristic of mild dysplasia are confined to the lower half of the epithelium, and with increasing grade, the atypical cells involve and replace the entire thickness of the epithelium. In CIS, no cellular maturation is observed on the epithelial surface.[3,4] Several series reported in the literature demonstrated that increasing grades of dysplasia were associated with increasing risk of developing ESCC.[5] Wang *et al.*[6] tried to identify the clinically relevant histological precursors of ESCC, analyzing a cohort of 682 endoscoped patients biopsied at baseline and followed for 13.5 years. The authors concluded that

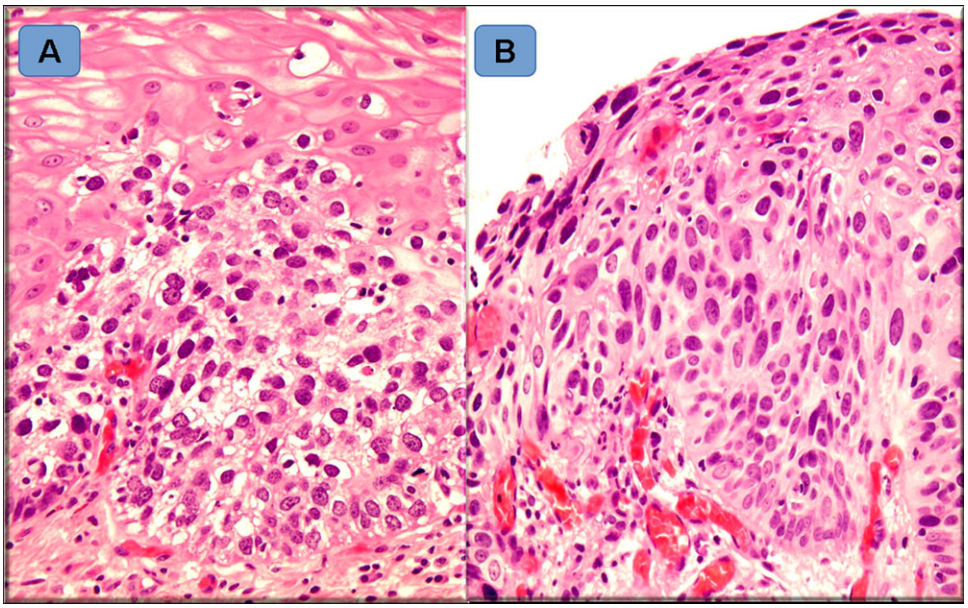

Figure 1. (A) Low- and (B) high-grade squamous intraepithelial neoplasia (hematoxylin–eosin, original magnification 40×).

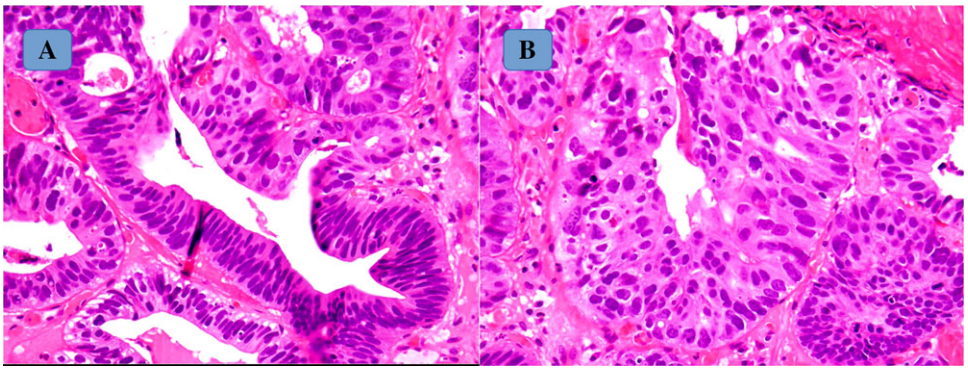

Figure 2. Low (A) and high (B) grade glandular dysplasia (hematoxylin–eosin, original magnification 40×).

squamous dysplasia and carcinoma *in situ* were the only histological lesions associated with a significantly increased risk of developing ESCC, and that increasing grades of dysplasia were strongly associated with increasing risk, indicating that the histological grading was clinically meaningful. In addition, recent advances in molecular biology[7] emphasize the strict sequence LG-IEN–HG-IEN in the development of invasive cancer, depending on genetic and epigenetic changes that parallel the histological modifications observed in the neoplastic progression. For example, Liu *et al.*[8] characterized the molecular events in the carcinogenesis of ESCC, analyzing precancerous and cancerous tissues resected from 34 esophageal cancer patients in which he evaluated the extent of LOH in 16 microsatellite markers on different chromosome regions. The authors found that the overall frequency of LOH at the 16 microsatellite loci was significantly increased as the pathological status of the resection specimens changed from LGD to HGD and ESCC, indicating that tumorigenesis of the esophageal squamous epithelia is a progressive process involving a series of molecular alterations. As the alterations accumulate to a certain degree, the cell morphology and behavior undergo a radical change, leading to malignancy. In our experience from 2000 to 2012, we detected 204 cases diagnosed as invasive ESCC. In 33 cases, we had available esophageal biopsies performed before the histological diagnosis of cancer; in all cases we detected the LG-IEN–HG-IEN sequence, emphasizing the concept that it is mandatory: a progressive passage from LGD to HGD in the progression towards invasive cancer.

2. Can low-grade glandular dysplasia give rise directly to invasive cancer?

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Dysplasia can be defined as an "unequivocal, neoplastic transformation of the epithelium excluding all reactive changes"[11] showing histological findings and expression of the DNA damage that precedes malignancy. On the basis of this definition, dysplasia must be considered a neoplastic process that cannot regress. In the context of BE, glandular dysplasia is then classified into two important categories, namely LGD and HGD, on the basis of accurate histological features (Fig. 2). LGD shows crypts with relatively preserved architecture or only minimal distortion, and stratified atypical nuclei (hyperchromatic, with an irregular contour and a dense chromatin) limited to the basal portion of the cell cytoplasm. With progression to HGD, the degree of cytological and architectural complexity becomes more advanced, with crypt budding, branching, and marked crowding. Cytologically, cells show marked nuclear pleomorphism and irregularity of contour, loss of cell polarity, and a higher number of atypical mitoses with a typical full-thickness nuclear stratification. The recognition of these pathological conditions depends mostly on an adequate biopsy sampling and an accurate diagnostic interpretation from the pathologist. First of all, a correct biopsy sampling is of paramount importance, mainly because of the "patchy" distribution of the dysplasia. Several authors suggest different types of sampling;[12,13] however, in our experience, at least one biopsy every

Table 2. Histological disease progression of Barrett's esophagus patients

Histological progression in BE	Dysplasia	Dysplasia + cancer
LGD	22	3
HGD	1	6
LGD + HGD	17	17

NOTE: In our experience, among 537 cases with histological diagnosis of BE between 2000 and 2012, during a follow-up period of 12 years, 72 patients showed histological progression of the disease. Of these, 40 patients developed dysplasia, while 26 patients developed dysplasia and subsequently cancer. The table shows in 17/40 (42.5%) patients with dysplasia the presence of the sequence LGD/HGD, while in 17/26 (65%) patients with dysplasia and subsequently cancer the presence of the sequence LGD/HGD/cancer.

2 cm in Barrett's area above the Z line must be obtained. Unfortunately, notwithstanding an adequate biopsy sampling, there is a high degree of subjectivity in diagnosis and grading of dysplasia in BE: Reid reported a 56–61% inter-observer agreement between categories named *negative/indefinite for dysplasia*, LGD/HGD, and *intramucosal carcinoma.*[14] Several series confirmed this problem,[15] emphasizing the concept that "accurate interpretation requires an experienced pathologist."[16] Nowadays, the existence of the sequence LGD–HGD–invasive cancer is also supported by IHC and molecular biology. In our experience, in particular, the application of immunohistochemical techniques revealed the existence of two pathways in the development of dysplasia in the context of BE: on one side, dysplastic cells show positivity for p16 and negativity for p53 and HER2; on the other side, dysplastic cells express p53 and HER2 and lack of p16 expression. These results reflect two different underlying molecular pathways. However, it is important to remember that "the results obtained with additional techniques must be interpreted with caution; the simple morphological recognition of dysplasia in endoscopic biopsies remains important for the management of the cancer risk" (Karel Geboes). We also demonstrated the existence of the strict sequence LGD–HGD–invasive cancer. We selected 537 cases with histological diagnosis of BE performed through an adequate biopsy sampling[13] between 2000 and 2012, analyzing for each patient all subsequent histological reports performed during a follow-up period of 12 years. Seventy-two patients showed histological progression of the disease; among these 40 patients developed dysplasia, while 26 patients developed dysplasia and subsequently cancer. Seventeen of 40 (42.5%) patients with dysplasia showed the presence of the sequence LGD–HGD, while 17/26 (65%) patients with dysplasia and subsequently cancer revealed the presence of the sequence LGD–HGD–cancer, emphasizing the concept that LGD cannot give rise directly to invasive cancer, but there is a morphological sequence LGD–HGD–cancer, confirmed also by IHC and molecular biology (Table 2).

3. Mutational spectra of Barrett's esophagus and adenocarcinoma

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Esophageal cancer is the eighth most common cancer worldwide and ranks as the sixth most common cause of cancer-related death owing to the late symptomatic presentation resulting in a low 5-year survival prognosis.[17]

EAC, the predominant subtype in developed countries, typically arises from glandular cells located in the distal one-third of the esophagus or gastroesophageal junction (GEJ), and is primarily associated with obesity, GERD, and BE. The incidence of EAC has rapidly increased over the past three decades, especially in developed countries, and is more common among Caucasian men over the age of 60 (Table 3). The rise in incidence rates is likely a secondary effect owing to an increase in the prevalence of obesity. However, the incidence rates vary widely from one area to another within the same country or ethnic group.[17] It is now widely accepted that EAC develops along a sequence of phenotypic and genetic alterations that evolve from metaplasia through dysplasia to carcinoma.

BE is the only well-recognized premalignant condition for the development of EAC. BE is defined as a metaplastic transformation occurring at the distal esophagus, where esophageal squamous epithelium is replaced by columnar epithelium with goblet cells. Patients with BE have a 30- to 60-fold higher risk of developing EAC than the general population; however, only 1–5% of these cases progress to dysplasia, and the risk of cancer increases significantly with the grade of dysplasia.[18]

Table 3. Adenocarcinoma of the esophagus: epidemiology, etiology, and symptoms

Age	Mostly > 60 years
Sex	Male dominant
Location	Distal one-third of the esophagus or gastroesophageal junction
Distribution	Western countries, middle or upper socioeconomic status
Risk factors	BE, GERD, obesity, tobacco smoking, lower esophageal sphincter–relaxing drugs, epidermal growth factor polymorphism, low intake of fiber, high intake of dietary fat and animal protein, nitrosative stress
Protective factors	*Helicobacter pylori* infection, aspirin, non-steroidal anti-inflammatory drugs (NSAIDs), cyclooxygenase-2 inhibitors, high consumption of fruit and vegetables, diets rich in fiber
Symptoms	Progressive dysphagia, odynophagia, halitosis, weight loss, chest pain

GERD is the major risk factor for BE. Patients with GERD have significantly more gastric acid and bile in their esophagus. The low pH, as well as the bile salts, induces expression of COX-2, which is a key enzyme of the arachidonic acid biosynthetic pathway. COX-2 catalyzes the conversion of arachidonic acid into various prostaglandins including PGE_2, which induces proliferation of BE cells and raises the probability for these cells to accumulate replication errors.[19] PGE_2 also inhibits tumor surveillance by inhibiting natural killer cell activity. COX-2 may also induce the production of ROS, resulting in oxidative stress and subsequent oxidative DNA damage that could contribute to carcinogenesis.[19]

The presence of genetic mutations is another important factor leading to EAC. The most common chromosomal alterations found in BE include allelic losses at chromosomes 4q, 5q, 9q, 18q, 7a, and 14q, as well as gains at 8q, 20q, 2q, 7q, 10q, 6p, 15q, and 17q.[20] Apart from gross chromosomal alterations, specific mutations in genes involved in cell cycle control (i.e., *p53*, *p16*, *Rb*, *cyclin D1*, and *c-myc*) have been reported. Accordingly, changes in the cell cycle of the affected epithelial cells, such as an increased number of cells in S phase (corresponding to DNA synthesis) and G_2 phase (pre-mitosis), have been described in BE patients.[21] Genomic instability in BE is also represented by widespread LOH, point mutations, alterations in microsatellite alleles, and epigenetic changes including hypermethylation of promoter regions in genes. Furthermore, polymorphisms of the epidermal growth factor (*EGF*) gene have been associated with higher serum levels of EGF and an increased risk of EAC, particularly in patients with BE.[22] The major events and genetic alterations associated with the development of BE and EAC are shown in Figure 3.

Obesity, which results from high caloric consumption and energy imbalance, appears to be a risk factor for the development of GERD and subsequent EAC.[23] How overnutrition leads to GERD development is still uncertain; however, obesity increases circulating levels of many steroid hormones and insulin-like growth factor-1 (IGF-1). The binding of IGF-1 to its receptor, IGF-1R, could transduce signals through several intracellular pathways, stimulating the malignant transformation of various epithelial and mesenchymal cells and protecting these cells from apoptosis.[19]

Tobacco smoking has been associated with a moderately increased risk of EAC, particularly in patients with BE. However, the strength of this association appears to be weak compared to the association with ESCC.[19] Antioxidants present in fruits and vegetables seem to have a protective effect, whereas low intake of fiber and high intake of dietary fat and animal protein appear to increase the risk of BE.[19] Exposure to nitroso compounds derived from dietary sources has also been associated with the development of EAC.[24]

Finally, some studies have suggested that *Helicobacter pylori* infection, and CagA strains in particular, may be associated with a protective effect against the development of BE and progression to EAC, probably by decreasing GERD.[19] The postulated mechanism is through the ability of *H. pylori* to induce atrophic gastritis, which likely increases intragastric ammonia production.[25]

Despite the wide knowledge in the etiology of esophageal cancer, the precise causes of EAC have not yet been identified, and the reasons for the trend of increasing EAC in Western countries remain largely unexplained. Nevertheless, some data suggest that interactions between risk factors may

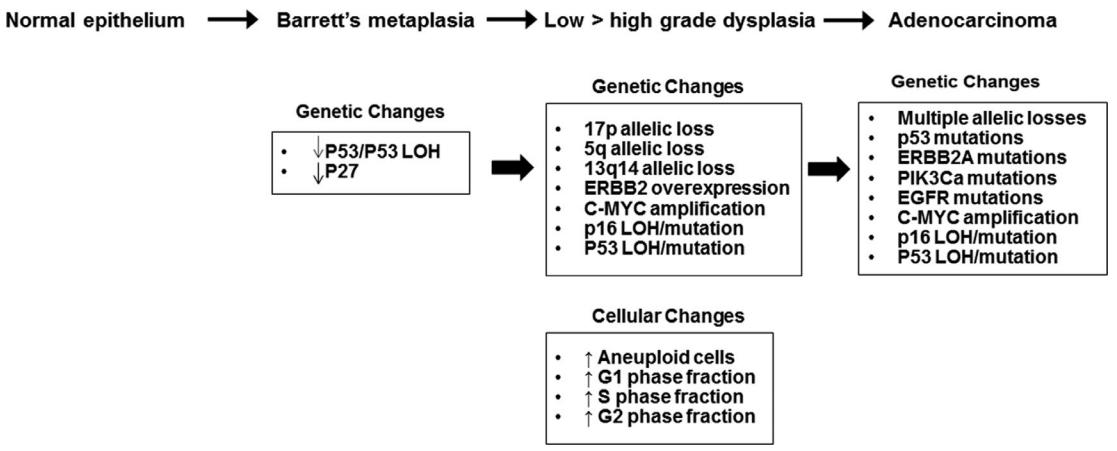

Figure 3. Temporal relationship between genetic alterations and the development of EAC. The succession of histopathological stages starting from normal epithelium and leading to adenocarcinoma is shown. Cellular alterations associated with these various stages are described in the boxes. The thick black arrows in the boxes signal increases in the S-phase fraction, the G-phase fraction, aneuploid cells, and the G2-phase fraction.

be more important in the development of EAC than the individual risk factors themselves.[26] Future directions include the need to stratify BE patients for esophageal cancer risk by the use of genetic susceptibility data and biomarkers, which would allow the identification of patients who are truly at risk for malignant generation in order to implement treatment and prevention strategies for these patients.

4. In Barrett's mucosa, is p53-immunoreactive low-grade dysplasia at higher risk than non-immunoreactive low-grade dysplasia for progressing to carcinoma?

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In order to answer this question, we have to answer two others. First, is the histologic diagnosis of LGD reproducible? Second, if it is reproducible, then is positive p53 staining of the dysplastic epithelium a better predictor of progression to carcinoma than negative staining?

In regard to the first question, the standard diagnoses used for biopsies of Barrett's mucosa include no dysplasia, LGD and HGD, invasive adenocarcinoma, and an indeterminate category referred as "indefinite for dysplasia," in which the pathologist viewing the case cannot decide if the changes are those of dysplasia or of regeneration. Unfortunately, the criteria for each diagnosis are not clear cut, so reproducibility of diagnoses may be poor. Certainly, something given the name of "indefinite for dysplasia" has a built-in lack of reproducibility. Several studies of dysplasia have found that reproducibility among even highly experienced pathologists is best at the highest end of the dysplastic spectrum, namely HGD and carcinoma, while it is poorest at the LGD end. Since there is no consistent definition of LGD, it is to be expected that the definitions in the literature and in the textbooks of gastrointestinal pathology will not be the same. The published definitions of LGD are filled with nonspecific, unhelpful words such as *slightly*, *reduced*, *larger*, *normal*, *mild*, *more atypical*, *irregular*, *inconspicuous*, and *marked decrease*.

In the Department of Pathology at the University of Michigan, three of us who handled most of the esophageal biopsy consultation cases independently classified the changes in every case. We found that agreement among the three of us in the diagnosis of LGD only occurred about one-fourth of the time. In contrast, we all agreed on the diagnosis of HGD 50% of the time, on carcinoma 75% of the time, and on non-dysplasia over 80% of the time.

In a study from the Netherlands, biopsies diagnosed by different pathologists in six community hospitals as LGD in 147 patients were reviewed by

two expert pathologists.[15] This raises an important issue, namely what qualifies a pathologist as an expert. There is no examination that certifies that a pathologist is expert in diagnosing LGD or any other epithelial change in Barrett's mucosa, or any other mucosa for that matter. Regardless, these two expert pathologists downgraded 85% of the LGD diagnoses to either negative or indefinite for dysplasia. For the 15% in which they confirmed the diagnosis of LGD, there was an 85% cumulative risk of progression to HGD or carcinoma in 109 months. In contrast, for the 85% of the cases that were downgraded, there was only a 5% risk of progression in 107 months. In another study from four U.S. centers covering 618 patients, 156 cases were diagnosed as LGD.[27] There was no central pathologist review of these cases, a problem with this study, because of the lack of reproducibility in the LGD diagnosis. Nevertheless, the authors found that progression to carcinoma from LGD was not significantly different in these 156 patients compared to the entire group of 618 patients. Unfortunately, these two studies are not totally comparable because they were not performed in the same way.

P53 is a tumor suppressor gene, a transcription factor important in cell cycle regulation. The encoded protein is nuclear, so the immunostain is easy to interpret. Several studies have looked at *p53* in Barrett's mucosa. In one study of 17 cases with *p53* mutations, there was a progressive increase in mutations from nondysplastic to LGD to HGD.[28] In another study, progression from indefinite and low-grade dysplasia correlated with positive p53 staining.[29] There was a progressive increase in staining percentage from negative to LGD to HGD, but in this study there was actually a decrease in staining in carcinomas. The authors felt that p53 positivity helped in the diagnosis of LGD. In another study, $p53^+$ LGD was much more likely progress than $p53^-$ LGD in 16 patients.[30] There are some problems with this study. All the cases were reviewed by three pathologists, but they all agreed on the LGD diagnosis in only four of the cases. Furthermore, the mean follow-up time for progressors was only 11 months, suggesting that at least some of the reported progressors actually had prevalent HGD or carcinoma rather than progression. In a 2013 study from the Netherlands covering 635 Barrett's patients, an increased rate of progression occurred in $p53^+$ LGD compared to $p53^-$ LGD.[31] However, in the same study, $p53^+$ nondysplastic epithelium also had an increased risk of progression.

Based on these somewhat disparate studies, it seems that patients with p53-immunoreactive LGD are at higher risk for progression than those with p53–non-immunoreactive LGD, although this is clouded by the lack of reproducibility for the LGD diagnosis. There is recent mention of p53 immunostaining being considered as an adjunct to routine histopathologic diagnosis, hoping to improve the diagnosis of dysplasia in Barrett's mucosa. As an example, in the latest Barrett's guidelines from the British Society of Gastroenterology, this recommendation is included: "The addition of a p53 immunostain to the histopathological assessment may improve the diagnostic reproducibility of a diagnosis of dysplasia in Barrett's oesophagus and should be considered as an adjunct to routine clinical diagnosis."[32] However, diagnoses of dysplasia are histologic diagnoses, not immunohistochemical diagnoses. Positive staining may improve recognition of those patients who are at risk to progress, but this may have nothing to do with the diagnosis of LGD.

5. Do lectins have a role in the progression to adenocarcinoma?

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Lectins are plant or animal proteins with high sensitivity and variable specificity for selected glycosyl moieties. These proteins are typically membrane bound and function locally as receptors. They can be employed as probes for glycosylated biomarkers in serum, cell fractions, and fresh or fixed tissue samples using chromatographic applications, immunofluoresence (when labeled with appropriate reporter molecules) and enzyme-linked histochemical detection systems (when biotinylated or when lectin-specific antibodies are used as secondary probes). An evolving understanding of the complex glycoprotein milieu in the normal and diseased esophagus paints an intriguing picture that suggests a re-emergent role for lectins in the diagnosis and management of BE and BE-associated EAC. This brief commentary will assess the value of quantitative serum glycomics to diagnosis and surveillance in BE patients and address the potential utility of fucosyl-, *N*-acetyl-D-glucosaminyl-, and *N*-acetyl-D-galactosaminyl-specific lectin

histochemistry in the evaluation of BE, both *ex vivo,* through analysis of standard formalin-fixed, paraffin-embedded tissue samples and *in vivo*, using lectin-based endoscopic visualization techniques.

Serum glycomics

The local production of glycoproteins in normal and diseased tissues is reflected in a complex spectrum of glycosylated molecules in serum. While it is unlikely that any single glycan in serum will be specific for a given tissue or disease, patterns of aberrant glycosylation in disease states may be disproportionately overrepresented in serum. Using a mass spectrometry–based evaluation of *N*-glycans that are released enzymatically from serum glycoprotein fractions obtained from patients with BE, BE with HGD, and BE-associated EAC, Mechref *et al.*[33] demonstrated by principal-component analysis that these patient populations can, to varying degrees, be separated from each other and from normal controls. Among the many glycosylation patterns identified, a limited subset of glycan structures reliably predicted the diagnosis of EAC when compared to controls. Similarly, BE–HGD could be distinguished from both controls and EAC, but in general with lesser accuracy. The differences between BE and BE–HGD were not diagnostically reliable, although BE glycomic profiles remained distinct from controls. The relevance of these differential profiles to biomarker discovery is a subject of ongoing investigation. Notably, the distinct repertoire of altered glycans from EAC was significantly depleted of fucosyl moieties when compared with controls, though many of the glycans of interest in this study contained core and terminal fucose residues. Interestingly, some of the latter may be of particular importance to the progression from BE through BE–HGD to EAC. Applying lectin-affinity chromatography to serum samples using the fucosyl-specific lectins *Aleuria aurantia* lectin (AAL) and *Lotus tetragonolobus* agglutinin (LTA), Mann *et al.*[34] identified a subset of proteoglycans that demand further scrutiny in this regard, including fetuin B, a cystatin that is somewhat overexpressed in BE–HGD relative to disease-free esophagus, but significantly increased in EAC; and elastin microfibril interface located protein 2 (EMILIN-2), an extracellular matrix glycoprotein that is involved in activation of the extrinsic pathway of apoptosis and inhibits cell growth. It is increased in BE–HGD and EAC when compared to control samples.

Lectin tissue histochemistry

The relative depletion of fucosyl residues in the BE to EAC sequence is immediately applicable to routinely processed biopsy samples. Initial studies by Bird-Lieberman *et al.*,[35] using a fucosyl-specific lectin derived from *Aspergillus oryzae* (AOL, a lectin-like AAL with broad specificity for fucosylated glycoproteins[36]), confirmed the expectation that decreased histochemical staining (measured as intensity and distribution of AOL staining in four cell compartments: global cell membranes, apical cell membranes, epithelial mucous globules, epithelial cytoplasm, and all membranes) may predict the transition from BE to EAC. Similar observations were made with the sialyl-selective wheat germ agglutinin (WGA, from *Tritium vulgaris*, a lectin that also selectively binds *N*-acetyl-D-glucosamine), though the relationship between loss of WGA staining and disease progression was less pronounced. These finding have led to the formulation of a robust risk-stratification biomarker panel for progression from BE to EAC that combines AOL stain results with LGD and DNA ploidy.[36] Among other lectins, the *N*-acetyl-D-galactosaminyl–selective agglutinin from *H. pomatia* (the edible Burgundy snail) selectively labels glycans that, when present or increased in number, may be associated with progression from locally invasive esophageal disease to metastasis.[37,38]

Endoscopic lectin-enhanced visualization of esophageal mucosa

Given the expectation that certain glycans will be decreased in BE, BE–HGD, and EAC compared to normal mucosa, selective lectin labeling of esophageal mucosa in BE patients may prove to be a useful guide for traditional biopsy techniques. Although a fucosyl-selective lectin would be ideal in this setting, work to date has employed WGA, in large part because this lectin is common in food products and thus carries minimal risk for topical or systemic adverse effects. Since glycomic studies suggest that sialyl residues are not significantly decreased in the progression from BE to EAC, the utility of WGA in this context is more likely due to its affinity for *N*-acetyl-D-glucosamine. As shown by Bird-Lieberman *et al.*,[39] the topical application of fluorescein-WGA readily defines areas of decreased emission when viewed endoscopically under UV

light, areas that reproducibly map to biopsy-proven BE–HGD and EAC. As an adjunct to confocal laser endomicroscopy,[40,41] lectin labeling could reasonably be expected to increase the efficiency of both scanning for and targeting of suspicious lesions, though this is, for the moment, an untested conjecture.

Conclusions

Serum glycomic analyses identify patterns of abnormal glycoprotein expression that distinguish adenocarcinoma patients from normal controls. Much of this difference may be due to selective loss of fucosylated glycans, an observation that has been successfully applied to tissue-based lectin histochemical studies of the progression from BE through BE–HGD to EAC. Fluorescently labeled lectins may also be useful probes for the endoscopic surveillance of BE, an intriguing observation that is potentially applicable to esophageal endomicroscopy.

6. Does racemase/AMACR immunoreactivity predict increased risk of esophageal adenocarcinoma in Barrett's esophagus?

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AMACR is present in many dysplasias and carcinomas, although it has been used histologically in the diagnosis of prostatic carcinoma owing to its high sensitivity and specificity (Fig. 4A). It also became apparent that dysplastic mucosa in ulcerative colitis may be AMACR positive with IHC[42] and that this may be of predictive value in determining patients at high risk[43] (Fig. 4B). Inevitably, the question was also reapplied to ask whether AMACR could be of similar value in identifying patients at highest risk for BE,[1] and a variety of patients suggested that it may be of value in intestinal-type dysplasia but likely not in foveolar dysplasia in BE[44–47] (Fig. 5). Its sensitivity came into question, as occasionally non-dysplastic mucosa would also stain (Fig. 5C). However, these largely lacked the power to answer the question.

However, in a study using the National Registry in the Netherlands,[48] among a cohort of 720 patients in the Barrett's registry, 635 patients were enrolled. Those with no neoplastic progression (586; 92%) were compared with those in whom neoplastic progression occurred (HGD or EAC in 49% or 8%, respectively). Those in the control group were endoscoped every 3 years, while those with LGD were endoscoped annually, undergoing a mean of four endoscopies over a mean of 6.6 years. AMACR IHC was performed on paraffin material of all surveillance endoscopies of patients who developed LGD, HGD, or EAC. In patients without dysplasia, AMACR IHC was performed on biopsies of a random surveillance endoscopy. Scoring was carried out on the worst biopsies, which were graded as 0, mild, or strong immunoreactivity.

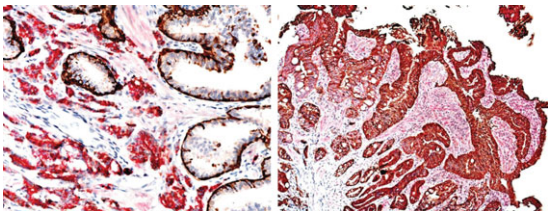

Figure 4. (A) Red staining AMACR immunoreactivity in prostatic carcinoma infiltrating between non-dysplastic prostatic glands highlighted by black-staining keratin. (B) Barrett's intramucosal carcinoma (top) staining uniformly red.

No AMACR expression was seen in 47% of the biopsy series, mild AMACR staining was seen in 48%, and 5% had strong immunoreactivity. Mild AMACR expression was seen in 46% of biopsy series without dysplasia, 53% of biopsy series with LGD,

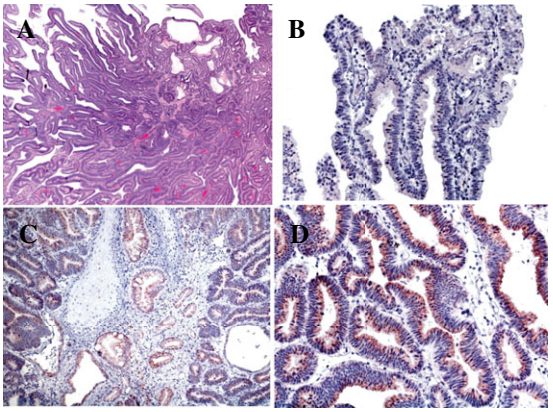

Figure 5. High-grade Barrett's dysplasia (hematoxylin–eosin). (B) foveolar dysplasia with no immunoreactivity. (C) Intestinal dysplasia with brown-staining AMACR immunoreactivity left and right, but also in the non-dysplastic mucosa (center). (D) Low-grade intestinal dysplasia showing brown-staining immunoreactivity with ACMACR antibody.

64% of biopsy series with HGD, and 57% of biopsy series with EAC. Strong AMACR expression was also seen in 3% of biopsy series without dysplasia, 10% of biopsy series with LGD, 27% of biopsy series with HGD, and 14% of biopsy series with EAC. Although mild AMACR expression was associated with a trend towards an increased risk of neoplastic progression (adjusted RR 1.6; 95% CI 0.9–3.1), the risk was especially elevated with strong AMACR expression (adjusted RR 4.8; 95% CI 1.9–12.6). After adjusting for histological diagnosis, only strong AMACR expression remained associated with a significantly increased risk of neoplastic progression (adjusted RR 1.4; 95% CI 0.8–2.6 and 3.3; 95% CI 1.3–8.4, respectively). The sensitivity of AMACR expression for predicting neoplastic progression was 67% with a specificity of 50%, PPV was 11%, and NPV was 97%. Of 41 (6%) patients with strong AMACR expression during follow-up, nine (22%) eventually developed HGD or EAC with an incidence rate of 7.1 per 100 patient years. The sensitivity of strong AMACR expression for predicting neoplastic progression was 10% with a specificity of 96%, a PPV of 22%, and a NPV of 93%.

These data make it abundantly clear that AMACR expression has a degree of predictability, but that it is not sensitive, and that non-dysplastic biopsies can have variable immunoreactivity. Once the cost of the test is taken into account, this limited value is not worth the cost of the test.

Conflicts of interest

The authors declare no conflicts of interest.

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