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Ductal metaplasia in esophageal submucosal glands is associated with inflammation and esophageal adenocarcinoma

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Abstract

Aims—Recent studies have suggested that esophageal submucosal gland (ESMG) ducts harbor progenitor cells that may contribute to esophageal metaplasia. Our objective was to determine if histologic differences exist between the ESMGs of individuals with and without esophageal adenocarcinoma (EAC).

Methods and Results—We performed histologic assessment of 343 unique ESMGs from 30 control patients, 24 patients with treatment-naïve high-grade columnar dysplasia (HGD) or EAC, and 23 non-EAC esophagectomy cases. A gastrointestinal pathologist assessed H&E stained ESMG images using a scoring system that assigns individual ESMG acini to five histologic types (mucous, serous, oncocytic, dilated, or ductal metaplastic). In our model, ductal metaplastic acini were more common in patients with HGD/EAC with 12.7% and 3.5% in controls ($p=0.006$). We also identified greater proportions of acini with dilation (21.9%, $p<0.001$) and, to a lesser extent,

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Author contributions:

Katherine S. Garman, Leandi Kruger, Marzena Swiderska-Syn and Shannon McCall performed the research. Katherine S. Garman, Anna Mae Diehl and Shannon McCall designed the research study. Anna Mae Diehl and Marzena Swiderska-Syn contributed essential reagents and tools. Katherine Garman, Samantha Thomas and Barry Moser analyzed the data. Katherine Garman and Shannon McCall wrote the paper with input from Barry Moser, Leandi Kruger, and Anna Mae Diehl and all authors reviewed the manuscript.

List of Online Supporting Information:

1. Details on Methods including: Selection of Controls, Gland Scoring, Data Integrity and Analysis Software, Statistical Analysis, Immunohistochemistry
2. Results of Inflammation Assessment in ESMGs
3. Discussion of Ductal Metaplasia in other organs
4. Supplemental Table 1 - Ductal Metaplasia in HGD/EAC Cases, Controls and non-EAC Esophagectomy Cases
5. Supplemental Figure 1 – CK7 in ESMG from autopsy control, in ESMG from a patient with EAC and in EAC.

ductal metaplasia (4.3%, $p=0.001$) in non-EAC esophagectomy cases versus controls. Ductal metaplasia tended to occur in areas of mucosal ulceration or tumor.

Conclusions—We found a clear association between ductal metaplastic ESMG acini and HGD/EAC. Non-EAC cases had dilated acini and some ductal dilation. Because ESMGs and ducts harbor putative progenitor cells, these associations could have significance for the understanding the pathogenesis of EAC.

Keywords

Esophagus; esophageal neoplasm; Barrett's Esophagus; ductal metaplasia; esophageal submucosal gland

Introduction

The overall five-year survival of esophageal adenocarcinoma (EAC) is a dismal 17%^{1, 2}, and incidence of EAC is increasing. Clinical guidelines promoting screening and surveillance for Barrett's esophagus (BE) represent an effort to improve long-term outcomes³. However, in part because the vast majority of patients who present with EAC have never been previously diagnosed with BE^{4, 5}, these guidelines have not resulted in an appreciable decrease in EAC². In addition, many resected EACs do not demonstrate a histologic background of BE, suggesting an alternative pre-neoplastic process^{6–9}.

The origin of esophageal progenitor cells remains an area of debate^{10–14}. One of the most intriguing theories focuses on esophageal submucosal glands (ESMGs), which occupy a protected position within the esophageal submucosa and connect to the mucosal layer via ducts. The role of ESMGs as a potential stem cell niche remains incompletely understood in part due to limitations of animal models. Mouse studies have demonstrated progenitor populations in the basal layer of squamous epithelium¹⁵, and at the squamo-columnar junction^{12, 14}. However, mouse esophagus does not contain ESMGs¹⁶, reducing the ability of the mouse to adequately model human esophageal biology. As a result, direct studies of human ESMGs are necessary in order to further our understanding of ESMGs in human esophageal disease.

ESMGs are a defining histologic feature of human esophagus^{17, 18, 19}. Autopsy studies have been used to evaluate ESMGs in normal esophagus while esophagectomy studies have focused on ESMGs in diseased esophagus^{20, 21}. Each ESMG is comprised of clusters of cells known as acini. Each acinus is surrounded by basement membrane and a myoepithelial cell layer. Human ESMG acini contain variable numbers of mucous cells, serous cells, and oncocytes^{22–24}. Mucous-producing cells demonstrate abundant clear to pale mucinous cytoplasm with small, basally-oriented nuclei and represent the predominant acinar cell type in ESMGs. Serous cells have abundant pale pink cytoplasm and produce neutral and sialated mucins as well as lysozyme and pepsinogen²⁴. Oncocytes are characterized by abundant bright pink cytoplasm consisting of densely packed mitochondria²².

In vivo, small ducts draining each acinus coalesce to form ducts of progressively greater diameter until a main duct is formed. Main gland ducts traverse the muscularis mucosa and

subepithelial connective tissue of the mucosa, allowing ESMGs to release their products into the esophageal lumen¹⁹. Duct cells within and near ESMGs appear cuboidal. As the lumen of the esophagus is approached, main duct cells transition to a squamous appearance.

Early investigations into ESMG ducts demonstrated continuity between ductal epithelium and neosquamous epithelium after squamous esophageal injury²⁵. Later reports described continuity between gland duct epithelium and a biphenotypic multilayered esophageal epithelium with both squamous and columnar features²⁶. Recently, ESMG ducts have been found in direct connection with both columnar BE as well as squamous islands occurring in a background of BE²⁷. However, the histologic changes occurring in the actual ESMG distal to the duct represent an area in need of further scientific investigation.

In our own practice, we noted ESMGs in esophagectomy specimens had a heterogeneous appearance. ESMGs from patients with EAC often lacked the classic appearance of predominantly mucinous acini. In order to quantify our observations, we systematically characterized the appearance of the ESMGs in patients with and without EAC or high grade dysplasia (HGD) in a blinded fashion. We used these data to test the hypothesis that the proportions of the various acinar types in ESMGs from patients with HGD/EAC differ from those found in ESMGs from patients without HGD/EAC.

Materials and Methods

Cases and Controls

Prior approval for this study was obtained from Duke University's Institutional Review Board. Using an existing database of patients who had undergone esophagectomy or esophagogastrectomy; cases from three years were selected for clinical and pathologic review. Cases (n=24) were included if the pathologic diagnosis was HGD/EAC. Because of well-characterized histologic changes occurring in patients after radiation therapy, patients who had received any thoracic cavity radiation therapy were excluded. Similarly, patients with neoadjuvant chemotherapy were excluded. Finally, cases were excluded if pathologic review of all available archival paraffin blocks failed to disclose 1 complete ESMG. All tumor sections were reviewed for confirmation of pathologic diagnosis.

For controls, we selected autopsy cases; further discussion of this choice of control is provided in Supporting Information. Autopsy files were sequentially reviewed. Controls (n=30) were included if the patient was 18 without clinical history or histologic finding of HGD/EAC. Standard histologic sections taken during autopsy at Duke include the anatomic gastroesophageal junction and the mid-tubular esophagus. All tubular esophagus sections and gastroesophageal junction sections containing squamous epithelium were reviewed. Controls were excluded if no ESMGs were identified in sections or if autolysis prevented detailed examination of ESMGs. One control sample was obtained from a surgical patient without BE or EAC who underwent esophagectomy for stricture. This group comprised our initial control group.

After the initial analysis of HGD/EAC cases and controls, an additional new comparison group (n=23) was formed from the esophagectomy cohort. The goal was to evaluate a

separate group of esophagectomy cases in order to assess a continuum of esophageal disease that included esophagitis and/or BE without HGD or EAC. We queried our 2000–2010 esophagectomy database for a group of cases without adenocarcinoma and without a history of neoadjuvant therapy. Diagnosis of achalasia and fistula were also excluded due to suspected anatomic distortion. From this group, 33 non-EAC esophagectomy cases were identified and tissue blocks were evaluated for presence of ESMGs. In ten cases, there were insufficient ESMGs; the remaining 23 non-EAC controls were available for scoring and analysis.

Clinical data collected for all esophagectomy cases included demographics, treatment history, and BE. Limited clinical data were available for autopsy cases and included basic demographics.

Autopsy and esophagectomy specimens had been fixed in 10% neutral buffered formalin and processed into paraffin blocks. Appropriate blocks were selected for preparation of research sections. Sections representing anatomic esophagus were identified; the anatomic esophagus was defined by either the presence of squamous epithelium or the presence of ESMGs, even if the overlying epithelium was columnar¹⁷. Five micrometer sections were cut from the blocks and used for standard hematoxylin and eosin (H&E) staining or immunohistochemical staining as described below.

ESMG Image Capture

A pathologist reviewed all slides to confirmation pathologic diagnosis, presence of autolysis, and presence of evaluable ESMGs using an Olympus BX-46 microscope. Subsequently, H&E-stained slides from selected paraffin blocks were examined using an Olympus IX71 microscope. An Olympus DP2-BSW digital camera and software was used to capture photomicrographs (Olympus Corporation, Tokyo, Japan). All ESMGs in case and control blocks were identified, and digital images were captured. Prior to scoring, each image was processed to obscure overlying epithelium. Blinded to clinical diagnosis, esophageal cancer status, and overlying esophageal epithelial type, a subspecialty fellowship-trained gastrointestinal pathologist evaluated isolated images of 343 ESMGs in the initial cases and controls.

Histologic Scoring System Developed

For quantification of acinar types, two investigators (KG, SM) devised an ESGM scoring schema (Table 1). The approach was based on the assumption that each ESGM acinus could be assigned to one of three types: mucinous (purely mucinous cells, no discernible lumen), serous (acinus containing any number of serous cells) or oncocytic (acinus containing any number of oncocytic cells). However, we noted the variable presence of a discernible lumen within some mucinous acini. Because normal ESGM are predominantly comprised of acini with tightly-clustered mucin-producing cells, the presence of a prominent discernible lumen within mucinous-cell lined acini was assigned a “dilated” category. We observed that while many dilated mucinous acini were lined by cells with a clear mucinous phenotype, some dilated acini appeared to be lined by cuboidal cells without histologic evidence of mucin production that appeared neither oncocytic nor serous. We assigned a fifth category of

putative “ductal metaplastic” based on these histologic features. More detailed description of acinar types is provided in Supporting Information.

In addition to assessment of acinar types, inflammation was subjectively graded for each ESMG unit. Inflammation was considered significant and scored if it was present around distal acini within the ESMG. Periductal inflammation was not considered significant²⁸. Inflammation grading used a typical pathologic scale of 0=none, 1=mild, 2=moderate, 3=marked.

Scoring was performed manually. The total number of acini per ESMG was counted. Acini were classified as one of the five types. Proportions were then calculated based upon total counts. The initial scoring analysis of cases and controls was performed on digital images processed to obscure overlying epithelium. Subsequently, the additional non-EAC esophagectomy cases were scored and HGD/EAC cases and the non-EAC esophagectomy cases were then evaluated to determine the type of epithelium overlying each ESMG.

Data Analysis

Study data were collected and managed using REDCap electronic data capture tools hosted at Duke University²⁹. Statistical Analysis was performed using SAS software, Version 9.3 of the SAS System for Windows, Copyright © 2002–2010 SAS Institute Inc. See supporting information for REDCap and SAS details as well as more detailed description of statistical analysis. The initial analysis compared HGD/EAC cases to controls. In brief, data were structured at the ESMG level per patient. Each patient possessed a variable number of ESMGs. For each ESMG, the number of acini was counted for each of five acinar types: mucinous, serous, oncocytic, dilated and ductal metaplastic. Proportions of acinar types within each ESMG were calculated. A general estimating equation (GEE) model that accounted for differences due to cases versus controls, acinar types, and the interaction of cases/controls and acinar types was used to calculate the predicted proportions of acini. Predicted proportions and interaction p-values are reported instead of odds ratios to allow the differences in acinar rates to be quantified. This type of model was selected for the analysis because it is designed to account for possible correlation caused by observing multiple glands and multiple acinar types per patient.³⁰ A description of this model (and justification for its selection over a multinomial model) are provided in supplemental material). Later, the same model was used to examine non-EAC esophagectomy cases versus HGD/EAC cases versus non-EAC controls.

Analysis of the association between proportion of ESMGs with different inflammation scores by group (HGD/EAC, non-EAC cases and controls) was performed using Chi-squared or Fisher’s exact test, as appropriate.

Immunohistochemistry

Cytokeratin 7 (CK7) faintly and peripherally stains normal ESMG mucous cells but stains ductal cells in a dense cytoplasmic pattern (Supplementary Figure 1). Esophageal squamous epithelium does not stain for CK7^{26, 31}. To verify the ductal metaplasia, we assessed CK7 in ESMGs and ducts (Dako Antibody M7018). Detailed methods are reported in Supporting Information. Pepsin retrieval was performed, protein blocking was performed, and then the

primary antibody was applied and incubated for 1 hour at room temperature. Antibody detection was performed with diaminobenzidine (Dako, Carpinteria, CA) and counterstaining was performed with hematoxylin solution and bluing agent.

Results

Patient Characteristics

The initial cohort included 54 patients: 24 cases and 30 controls. The cases had undergone esophagectomy or esophagogastrectomy for a diagnosis of HGD (n=7) or EAC (n=17). Of the 24 EAC/HGD cases, 21 (87.5%) had histologic evidence of BE. As per exclusion criteria, none of the 24 patients with HGD/EAC had previously received chemotherapy or thoracic radiation. All control patients were without HGD/EAC; no control patients had BE. After the initial analysis, an additional non-EAC esophagectomy comparison group was added consisting of 23 diseased esophagus (without adenocarcinoma) but affected by other esophageal conditions. Diagnoses in this group included esophagitis (n=7), esophageal squamous cell cancer (ESCC) (n=10, 2 with BE), paraesophageal hernia (n=2), leiomyoma (n=2, 1 with BE), liposarcoma (n=1).

As expected, the cohort of EAC/HGD cases tended to be male (87.5%). In comparison, gender in the control group and non-EAC esophagectomy group were more balanced (53.3% and 52.2% male respectively). Median age of the HGD/EAC cases was 66 years (range 38–over age 80). This was similar to controls with a median age of 64 years (range 26–over age 90). The non-EAC esophagectomy comparison group had a mean age of 61 years (range 27–77).

ESMG Histology for cases and controls

An average of 1.2 esophageal blocks containing ESMGs was identified for review from both cases and controls (range 1–2 in cases and range 1–4 in controls). Histology of all 343 ESMGs identified was reviewed. The data analyzed were complex with variable numbers of ESMGs per patient: ESMG counts ranged from 1 to 24 with a total of 147 ESMGs for cases (mean 6.1) and 196 ESMGs for controls (mean 6.5). The number of acini within each ESMG ranged from 1 to 320. As expected, the most common type of acinus within the ESMGs was the mucinous acinus, comprised solely of mucous-producing cells clustered tightly without a discernible lumen (Figure 1). In addition to mucinous acini, oncocyctic acini (containing oncocyctic cells as in Figure 2) and serous acini (containing serous cells) were variably present. We also identified a dilated mucinous (with a central lumen) type of acinus, and found histologic evidence suggesting ductal metaplasia within some ESMGs (defined in Table 1 and presented in Figure 3). Dense and diffuse cytoplasmic positivity for CK7 confirmed ductal phenotype. Mucinous acini express CK7; it is generally peripheral and faint. Representative CK7 staining is presented in Figure 3 and in Supplemental Figure 1.

Proportions of dilated acini, acini with ductal metaplasia, and oncocyctic acini within ESMG varied significantly between the HGD/EAC cases and controls. While there was no substantial difference in the proportion of serous acini between controls and cases (10.8%

and 7.9%, $p=0.37$), the proportion of dilated mucinous acini in HGD/EAC cases was almost twice as high as in controls (11.6% to 5.8%, $p=0.05$) and the proportion of acini with ductular metaplasia was 3.6 times as high in HGD/EAC cases as in controls (12.7% to 3.5%, $p=0.006$). The proportion of oncocyctic acini was lower in HGD/EAC patients when compared to controls (1.0% to 7.9%, $p=0.005$). Predicted proportions of acinar types for HGD/EAC cases and controls are presented in Table 2 along with results for an additional group of non-EAC esophagectomy cases that was added to the analysis in order to consider the relationship of ductal metaplasia to mucosal inflammation. The non-EAC esophagectomy group is described in more detail later in the results section.

The grade of inflammation within ESMGs correlated with proportions of acinar types. Inflammation scores of ESMG spanned the range of 0 (none) to 3 (marked) although marked inflammation was only present in 9 ESMGs in the cases (6.1%) and 1 ESMG in the controls (0.5%). Examples of ESMG inflammation grades are presented in Figure 4. Overall, 14 cases (58.3%) had at least one ESMG with grade 2 (moderate) or grade 3 (marked) inflammation compared to eight controls (26.7%). Mean proportions of ESMGs by inflammation grade are presented in Table 3 **for cases, controls, and non-EAC esophagectomy cases**. ESMGs with any amount of inflammation (ESMG inflammation grade higher than zero) were associated with higher proportions of all four non-mucinous acinar types (dilated, ductal metaplasia, oncocyctic, and serous). The correlation between non-mucinous acinar types was stronger with higher grades of inflammation as for grade 2 versus grade 0 (p -value = 0.03). For all grades of ESMG inflammation, proportions of dilated acini and acini with ductal metaplasia were smaller for controls than for HGD/EAC cases and the proportions of oncocyctic and serous acini were larger for controls than HGD/EAC cases. Including grade of ESMG inflammation in the model heightened overall differences between HGD/EAC cases and controls, data not shown.

Increased patient age was also associated with increased proportion of non-mucinous acinar types (dilated ductal, oncocyctic and serous), ($p=0.006$) in HGD/EAC cases versus controls. When age was added to a model that included cases versus controls, acinar types, and the interaction of these two factors, there continued to be more dilated acini and ductal metaplasia in the HGD/EAC cases versus the controls, and there were fewer oncocyctic and serous acini in HGD/EAC cases versus controls. Furthermore, as age increased, the size of the differences between these proportions also increased, data not shown. When the model was used to evaluate the association between presence or absence of BE in the HGD/EAC cases and acinar types within ESMGs, presence of BE in the cases did not exert much effect ($p=0.88$).

An additional group of esophagectomy patients was added to the study following the initial analysis. This new group was added in order to evaluate ESMGs associated with non-EAC esophageal diseases. The new non-EAC esophagectomy group was heterogenous, including cases of esophagectomy performed for ulcers and intractable inflammation. In these cases, pathologists were responsible for carefully excluding cancer. As a result, these non-EAC esophagectomy cases tended to have many blocks of esophageal tissue available for clinical evaluation. Compared to the cases and controls (with mean 1.2 blocks per case with ESMGs), the non-EAC esophagectomy group had an average of 5.1 blocks per case with

ESMGs (range 1–26). The total glands evaluated for the non-EAC esophagectomy cases was 698 (mean 30.3 per case, range 1–178).

Non-EAC esophagectomy cases had the highest proportion of dilated mucinous acini (21.9%) compared to EAC cases (11.6%, p-value <0.001) and controls (5.8%, p-value <0.001). While non-EAC esophagectomy cases also had more ductal metaplasia (4.3%) than was found in the control group (3.5%, p-value 0.001), there was not a significant difference from HGD/EAC group (12.7%, p-value 0.19); these results are presented in Table 2. Supplemental Table 1 provides unadjusted counts for all three groups: HGD/EAC cases, non-EAC esophagectomy, and controls.

An analysis of the overlying epithelium was also performed for the HGD/EAC cases as well as the new non-EAC esophagectomy cases. As per the initial study-design, the overlying epithelium in the control group was entirely squamous. Table 4 provides data on frequency of various types of overlying epithelium found over ESGMs in the esophagectomy cases with HGD/EAC and the non-EAC esophagectomy cases. Mean unadjusted proportions of ductal metaplasia for ESGMs with different types of overlying epithelia is reported in Table 4. Overall, ESGMs underlying EAC, ulcer, or SCC frequently contained ductal metaplasia. Very acute injury such as perforation with peritonitis was not associated with increased proportions of ductal metaplasia in ESGMs. BE was much less commonly found overlying ESGMs than was squamous epithelium. Interestingly, in the HGD/EAC cases, there were many instances of squamous islands identified overlying the ESGMs.

Discussion

Our results provide the first evidence that proportions of ESMG acinar types differ between HGD/EAC patients and autopsy controls, and specifically that increased ductal metaplasia in ESGMs is seen in patients with HGD/EAC. There is a collection of studies demonstrating continuity between ESMG ducts and squamous islands, multilayered (biphenotypic) esophageal epithelium, and/or BE itself^{26,27}. However our study is one of the first to examine the ESMG themselves³², and our study provides a scoring schema for quantitative ESMG assessment that will be useful as these investigations continue.

In some patients with BE, ESMG ducts and overlying metaplastic epithelia have been described as sharing a common mutation in the tumor suppressor gene p16, suggesting an association between the underlying ESMG ducts and overlying BE³¹. In addition, when mitochondrial mutations in the cytochrome C oxidase gene were used to map the clonal origins of neoplastic esophageal epithelial cells, a common mitochondrial gene mutation was also found in both the submucosa and the surface epithelium³³. This suggests that investigations of esophageal neoplasia should include not only the ESMG ducts, but the ESGMs themselves.

Our results support the concept that ESGMs themselves may undergo metaplasia, particularly in the setting of ESMG inflammation. To illustrate this concept, we propose a possible schema of changes in ESGMs (i.e., loss of acinar type variability, dilation of

mucinous acini, ductal metaplasia of mucinous acini) that may be associated with changes in the overlying mucosa occurring during the evolution of HGD/EAC in Figure 5.

A recent paper by Braxton et al described ESMGs with a process previously reported in minor salivary glands called “necrotizing sialometaplasia.”³² This was defined as replacement of normal ESMG acini with duct-like structures. This group also reported a different process where normal ESMGs are replaced with “oncocytic glandular metaplasia.” Using an esophagectomy database divided into those with and without BE, their group reported an association of both abnormal ESMG phenotypes with BE; 22% of non-BE-controls had metaplasia compared to 56% of BE cases.

Our study supports the novel concept that abnormal ESMG phenotypes are associated with abnormal esophageal epithelial phenotypes. Both the Braxton study and our analysis note the ductal metaplastic change associated with EAC. However, there are several important differences between our two studies. In this current investigation, we focused on ESMGs associated with HGD/EAC versus ESMGs associated with relatively normal esophagus controls. We specifically excluded patients with radiation and/or chemotherapy in an effort to eliminate the possible effects of these therapies on ESMG phenotype. Rather than assigning ESMG phenotypic changes into broad categories such as necrotizing sialometaplasia and oncocytic glandular metaplasia, we developed a quantitative schema for assessing individual acini within ESMGs.

Our additional analysis of non-EAC esophagectomy cases establishes that ESMG ductal metaplasia is also present in the setting of active esophageal inflammation (such as esophageal ulceration). In other organs, duct-like progenitors have been reported^{34, 35}. We hypothesize that in ESMGs, the ductal metaplasia associated with inflammation may persist in some instances, and a signaling climate may develop to support and foster the development of EAC. Interestingly, ductal metaplasia was very rarely identified in ESMGs underlying squamous islands in patients in this group. Model systems are needed to prospectively study the association we report between ductal metaplasia in ESMGs and esophageal ulcer.

This study was not designed to assess the association between ESMGs and esophageal squamous cell carcinoma. However, in the few cases of esophageal squamous cell cancer evaluated as part of the non-EAC esophagectomy cohort, we were surprised to find some ductal metaplasia. We noted a few cases of squamoid metaplasia in the ducts of patients with esophageal squamous cell carcinoma, and we propose further research in this area to more fully evaluate this finding.

The ductal metaplasia we identified in ESMGs is consistent with previous studies that suggest association of expanded ductular compartment with inflammation and neoplasia in other organs such as pancreas^{36,37}. A more detailed discussion of supporting studies is provided in Supporting Information. The association between ductal metaplasia in ESMGs and EAC is further supported by case reports of EAC developing within an ESMG³⁸. Our data present strong evidence to support future research in this area.

The retrospective nature of our study presents inherent limitations. Previous research demonstrated heterogeneous distribution of ESMGs within the human tubular esophagus³⁹. The esophagus could not be fully mapped and evaluated for ESMGs as only selected blocks were available for review, however the average number of slides for cases and controls did not differ. The work presented here justifies future prospective studies in autopsy and esophagectomy cases for more complete mapping of ESMGs including relationship to BE segments and distribution of ESMGs with phenotypic changes within the esophagus.

Identifying ductal metaplasia in ESMGs may have important clinical implications. Dysplastic BE may be treated with surgery, endoscopic mucosal resection, or ablation^{40,41}. However, of those who respond to radiofrequency ablation (RFA), in two years post-ablation, BE subsequently re-emerges in 33%⁴². It is possible that persistent abnormalities in the ESMG compartment could explain failure to maintain normal squamous epithelium following ablative mucosal therapies.

We did not identify an independent association between BE and ductal metaplasia in ESMGs. However, our study was not designed to evaluate this association. Because our cohort of cases was comprised of advanced disease (in which short segments of BE could be replaced by cancer), it is not possible to determine if ductal metaplasia in ESMGs precedes development of HGD/EAC. To further evaluate the presence of ductal metaplasia in BE, additional studies are required. Endoscopic mucosal resection cases may provide further opportunity for such research.

Endoscopic mucosal resection (EMR) and endoscopic mucosal dissection (EMD) remove both the esophageal mucosa and the esophageal submucosa. As EMR/EMD become more common, pathologists will have the opportunity to evaluate more ESMGs. We have provided a basic framework for scoring ESMG inflammation and quantifying acinar types within the ESMGs. In this study, scoring was performed with non-automated (manual) counting of each acinar type. In future, it may be beneficial to develop automated scoring based upon image analysis software. There is an opportunity and a need for prospective validation of the dilated and ductal metaplastic acinar phenotypes in both BE and EAC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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IRB Approval was obtained for this work through two protocols: Esophagectomy Database Protocol # 39682 (initial approval 07/31/2012) and Immunohistochemistry #19479 (initial approval/exemption 09/02/2009).

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Figure 1.

Normal-appearing ESMG from a control with no history of esophageal cancer: A. A 40× magnification image with squamous epithelium is present, and an esophageal duct is seen reaching in the direction of the ESMG to the lumen of the esophagus. This ESMG demonstrates predominantly non-dilated mucinous acini and there is no evidence of inflammation in this ESMG. B. 200× magnification demonstrates normal appearing mucinous acini without discernible lumens. C. Serous cell with light pink cytoplasm in a different ESMG from the same autopsy case (arrow).

Figure 2.

ESMGs containing oncocytic acini from controls: Oncocytes appear densely eosinophilic with a centrally located nucleus. A. 100 $\times$ view of an ESMG containing 70% oncocytic acini, present on the left (arrows). Mucinous acini, characterized by their pale mucosa are present right side of this ESMG. B. 200 $\times$ view of oncocytes from Panel A. C. 40 $\times$ view of ESMG that contains 25% oncocytic acini. D. 40 $\times$ view of ESMG containing 62% oncocytic acini.

Figure 3.

Dilated acinar phenotype (A, B, D, E) and Ductal metaplasia (C and F). A. 40× view of H&E of ESGM from a patient with BE with high-grade dysplasia; 88% of the acini were scored as dilated. B. 100× view of same ESGM with H&E illustrating dilated mucinous glands. C. 100× view of ESGM from an EAC patient with 84% ductal metaplastic acini and prominent inflammation. D. 40× view of CK7 staining illustrating contrast between dilated mucinous glands and more densely staining ducts of ESGM shown in panel A. E. 100× view of same ESGM illustrating dilated mucinous glands with CK7 staining; the arrow in panel E indicates normal CK7 pattern in dilated mucinous acini.. F. 100× view of CK7 staining of ductal metaplasia and inflammation within the ESGMs shown in Panel C; the arrow in panel F indicates ductal pattern of prominent cytoplasmic CK7 staining. Both patients had BE with high-grade dysplasia and EAC and both were treatment naive (no radiation or chemotherapy) prior to esophagectomy.

Figure 4.

Examples of different grades (0–3) of ESGM inflammation: Numbers on the images represent the ESGM inflammation score. Arrows indicate inflammatory infiltrate within ESGMs. As shown in panel 3, lymphoid follicle formation is designated grade 3 (arrow).

Figure 5.

Proposed progression of ESMG from normal, development of ductal dilation and then ductal metaplasia. A. Normal ESMG B. ESMG with ductal dilation and early ductal metaplasia C. Ductal metaplasia

Table 1
ESMG scoring system for inflammation and acinar type

The left column provides categories to score overall inflammation within ESGM. The second column scores different acinar types (as defined in column three) with by counting acinar types present in an ESGM. Each acinus within an ESGM should only be assigned one category. The bottom cell of this column should represent the total tally of the five different acinar types and should equal all acini within an ESGM. The far right column allows calculation of percentages of each acinar type per ESGM.

Scoring of Each ESGM	Types of Acini (score below)	Definitions of Acinar Types	% Each Acinar Type of Total # Acinar Units
Overall inflammation (score below)	# Mucinous	Pale, plump, glandular cells	
0 = none	# Serous	Small dense granules and peripherally located nucleus	
1 = mild	# Oncocytic	Eosinophilic (mitochondria-rich) with central nucleus	
2 = moderate	# Dilated Mucinous	Pale mucinous cells with dilated lumen	
3 = marked	# Ductular Metaplasia	Ductal-type epithelium with dilated lumen	
	Total # of Acinar Units	Calculated as sum of five different acinar types	Should add to 100%

Table 2
Predicted proportions of acinar types for HGD/EAC cases, controls, and the non-EAC esophagectomy group

Proportions of acinar types were predicted using a general estimating equation model that controlled for the repeated measurements of the variable number of ESMGs per subject. Predicted proportions of four acinar types are presented for the HGD/EAC cases, the autopsy controls and the non-EAC esophagectomy group. P-values are provided for the comparisons between groups by acinar type.

	HGD/EAC Cases	Controls	Non-EAC	P-Values		
				EAC vs. Controls	Non-EAC vs. Controls	Non-EAC vs. EAC
Dilated	11.6%	5.8%	21.9%	0.05	<0.001	<0.001
Ductular Metaplasia	12.7%	3.5%	4.3%	0.006	0.001	0.19
Oncocytic	1.0%	6.7%	2.6%	0.005	0.19	0.01
Serous	7.9%	10.8%	0.9%	reference	reference	reference

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Table 3
Proportion of ESMGs with Inflammation Grades 0–3 for Cases (N=147 ESMGs) and Controls (N=195 ESMGs) and non-EAC (N=695 ESMGs)

The proportion of ESMGs with each category of inflammation Grades 0–3 as scored (where 0=none, 1=mild, 2=moderate, 3=marked) was calculated for both cases (EAC and non-EAC) and controls. Chi-squared or Fisher’s Exact Tests, as appropriate, were used to compare the proportions between Non-EAC cases, EAC cases, and controls.

Grade	HGD/EAC Cases	Controls	Non-EAC Esophagectomy	P-Values
0	33 (22.5%)	59 (30.3%)	434 (62.5%)	<0.001
1	84 (57.1%)	125 (64.1%)	206 (29.6%)	<0.001
2	21 (14.3%)	10 (5.1%)	46 (6.6%)	0.002
3	9 (6.1%)	1 (0.5%)	9 (1.3%)	<0.001

Table 4
Types of esophageal epithelium directly over ESMGs and amount of ductal metaplasia associated with epithelial types

Type of epithelium overlying each ESMG was assessed after the initial blinded scoring. In HGD/EAC cases, there were several instances where a squamous island was associated with ESMG. Those ESMGs had low-levels of ductal metaplasia. In contrast, HGD/EAC as well as SCC and ulcer were associated with ESMGs in the submucosa. Overlying epithelium could not be determined for a small number of ESMGs in the HGD/EAC group.

EAC (143 ESMGs)				Non-EAC (698 ESMGs)			
	# ESMGs	Mean proportion ductal metaplasia in ESMGs			# ESMGs	Mean proportion ductal metaplasia in ESMGs	
Squamous epithelium	74	15.6%		Squamous epithelium	551	10.8%	
Squamous island	17	5.4%					
BE	28	11.8%		BE	8	26.9%	
Columnar	11	20.9%					
EAC	13	37.3%		SCC	58	28.3%	
				Gastroesophageal Junction	36	8.5%	
				Ulcer	11	54.4%	
				Peritonitis	34	5.0%	