Ann. N.Y. Acad. Sci. ISSN 0077-8923

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Special Issue: *Global Perspectives on Esophageal Diseases*
REVIEW

The microscopic anatomy of the esophagus including the individual layers, specialized tissues, and unique components and their responses to injury

Xuchen Zhang,[1] Deepa Patil,[2] Robert D. Odze,[3,4] Lei Zhao,[3,4] Mikhail Lisovsky,[5] Maha Guindi,[6] Robert Riddell,[7,8] Andrew Bellizzi,[9] Rhonda K. Yantiss,[10] Ilke Nalbantoglu,[1] and Henry D. Appelman[11]

[1]Department of Pathology, Yale University, New Haven, Connecticut. [2]Department of Pathology, Cleveland Clinic, Cleveland, Ohio. [3]Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts. [4]Department of Pathology, Harvard University, Boston, Massachusetts. [5]Department of Pathology, Dartmouth University, Lebanon, New Hampshire. [6]Department of Pathology and Laboratory Medicine, Cedars-Sinai Hospital, Los Angeles, California. [7]Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario. [8]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario. [9]Department of Pathology, University of Iowa, Iowa City, Iowa. [10]Department of Pathology and Laboratory Medicine, Weill Cornell Medicine, New York, New York. [11]Department of Pathology, University of Michigan, Ann Arbor, Michigan

Address for correspondence: Henry D. Appelman, M.D., Department of Pathology, University of Michigan, M5220 Medical Science Bldg 1, 1301 Catherine Street, Ann Arbor, Michigan 48109. appelman@umich.edu

The esophagus, a straight tube that connects the pharynx to the stomach, has the complex architecture common to the rest of the gastrointestinal tract with special differences that relate to its function as a conduit of ingested substances. For instance, it has submucosal glands that are unique and have a specific protective function. It has a squamous lining that exists nowhere else in the gut except the anus and it has a different submucosal nerve plexus when compared to the stomach and intestines. All of the layers of the esophageal wall and the specialized structures including blood and lymphatic vessels and nerves have specific responses to injury. The esophagus also has unique features such as patches of gastric mucosa called inlet patches at the very proximal part and it has a special sphincter mechanism at the most distal aspect. This review covers the normal microscopic anatomy of the esophagus and the patterns of reaction to stress and injury of each layer and each special structure.

Keywords: esophagus; anatomy; injury response

Introduction

The esophagus is a straight tube connecting the mouth to the stomach. It has the same layers found in the rest of the gastrointestinal tract, with the mucosa on the inside and the muscularis propria (MP) on the outside, blood and lymphatic vessels, and nerves, yet it has a unique job and a unique set of diseases. This review analyzes the published information on all these layers and structures, concentrating on their normal microscopic anatomy and common reactions to injury. In addition, there is detailed analysis of two unique esophageal strictures, the inlet patch and the lower esophageal sphincter (LES).

All authors contributed equally to the manuscript.

How does the esophagus evolve into the normal human adult structure?

The esophagus is a 23- to 25-cm musculomembranous tube that begins at the cricoid cartilage, passes through the thorax within the posterior mediastinum, and extends several centimeters below the diaphragm to the gastroesophageal junction (GEJ). In practice, clinicians use the incisor teeth as a landmark: the endoscopic distance from the incisor teeth to the GEJ is approximately 40 cm in adults, but it may vary from 30 to 43 cm. The normal esophageal

doi: 10.1111/nyas.13705

mucosa is lined by stratified nonkeratinized squamous mucosa. The lamina propria (LP) is composed of loose connective tissue that contains mucous glands in the distal portion. The esophageal muscularis mucosae (MM) are composed of longitudinally organized smooth muscle. The submucosa (SM) consists of irregular connective tissue that contains the larger vascular and lymphatic vessels, nerve fibers, and mucous glands with their ducts open into the esophageal lumen. The MP is composed of striated muscle in the upper part, smooth muscle in the lower part, and a mixture of the two in the middle. The myenteric plexus (Auerbach's plexus) is present between the muscle layers. The esophagus lacks a serosal layer, except for its most distal portion.

The primary function of the esophagus is to serve as a passage for food and liquid from the pharynx to the stomach. Although this process sounds straightforward, it is fraught with multiple barriers to its success. As described above, the esophagus is ensheathed by layers of muscles that are essential to generate peristalsis to move food. The thick stratified squamous epithelium of the mucosa is required to sustain the passing of the abrasive raw food, which is facilitated by secretions of the esophageal submucosal glands. However, the embryonic esophagus is initially lined with a simple columnar epithelial layer instead of stratified squamous. The human esophagus begins to form during the fourth week of embryonic development with the formation of the foregut, a structure that also gives rise to other organs including the trachea, lung, and stomach. Separation of the esophagus from the tracheal tube and transition of epithelial lining from columnar to squamous epithelium are the two major developmental processes. During embryonic development, the esophagus and trachea initially share a single-lumen tube at the anterior region of the foregut. Lateral grooves invaginate on each side of the proximal foregut and fuse creating the tracheoesophageal septum. The septum separates the tracheal tube and esophagus and generates the trachea ventrally and the esophagus dorsally. This tracheal–esophageal separation occurs at approximately 4–6 weeks of gestation in humans.[1] The failure of this process results in various anomalies such as esophageal atresia with or without tracheoesophageal fistula (EA/TEF).

It is well recognized that the separation of the esophagus from the tracheal tube is regulated by diverse signaling crosstalk between the epithelial cells and surrounding mesenchyme, which are highly coordinated by transcriptional factors and signaling pathways.[2,3] Specifically, the dorsal foregut endoderm expressing Sox2 gives rise to the esophagus, while the ventral foregut endoderm expressing the transcription factor Nkx2.1 forms the trachea. Reciprocal inhibition occurs between *Sox2* and *Nkx2.1.* Both *Sox2* and *Nkx2.1* are crucial factors involved in foregut separation and columnar to squamous epithelium transition. Nkx2.1-null mice exhibit incomplete foregut separation, resulting in a condition similar to tracheal agenesis, known as complete tracheoesophageal cleft. Similarly, downregulation of Sox2 in the early foregut leads to EA/TEF.[4] The functions of *Sox2* and *Nkx2.1* are regulated by several signaling pathways.[1,5] WNT/β-catenin signaling pathway plays a crucial role in specifying $Nkx2.1^+$ respiratory endoderm progenitors during development. Wnt2 and Wnt2b are expressed in the ventral anterior mesoderm surrounding the region of the anterior foregut endoderm, where $Nkx2.1^+$ respiratory endoderm progenitors are located. Embryos lacking Wnt2/2b expression exhibit complete lung agenesis and do not express Nkx2.1. This phenotype is recapitulated by an endoderm-restricted deletion of the β-catenin gene. The ability of Wnt/β-catenin signaling to promote $Nkx2.1^+$ respiratory endoderm progenitor fate is dependent upon other associated signaling pathways, such as bone morphogenetic protein (BMP) signaling.[6] Loss of BMP signaling in the foregut endoderm through inactivation of the BMP receptors (BMPR1a/1b) leads to tracheal agenesis. BMP signaling appears to act by repressing Sox2, which allows for expression of Nkx2.1 in the presumptive lung endoderm.

When the esophagus is completely separated from the trachea in the 4–6 weeks of gestation, the esophageal epithelium appears as a pseudostratified columnar epithelium, which then becomes to ciliated near the mid-esophagus at 8 weeks of gestation. Starting from the fourth month of gestation, the ciliated epithelium gradually transits to squamous epithelium bidirectionally until a nonkeratinized stratified squamous epithelium is fully developed. Residual islands of columnar epithelium remain as inlet patches or grow down to generate submucosal mucous glands. Meanwhile, the mesenchymal cells surrounding the nascent esophagus proliferate and differentiate into muscularis mucosa and

the MP, with networks of blood vessels and nerves running throughout. Although controversies remain regarding the cellular origin of striated muscle and regulation of esophageal muscular development, the use of genetic mouse models has revealed that multiple genes, transcription factors, and signal pathways are involved in this process.[1,7] Specifically, the cell surface receptor Cdo is required for setting up the striated-smooth muscle boundary. The bHLH transcription factor Myf5 is required for striated muscle differentiation. Homeobox transcription factors Foxp1 and Foxp2 are important for striated muscle development. Mutant mice lacking Foxp2 on a Foxp1 heterozygous background completely lose striated muscle. Deletion of the Wnt signaling receptor Fz4 also affects the formation of striated muscle, leading to esophageal distension. Moreover, *Pax7* mutant mice develop megaesophagus due to the disrupted differentiation of striated muscle and abnormal orientation of smooth muscles.[8] Similar to the process of esophageal separation and muscular development, many transcriptional factors and signaling pathways are involved in the process of esophageal columnar to squamous epithelium transition. Opposite to its expression during tracheal and lung development, Sox2 remains highly expressed and is required for the stratification and lineage differentiation of the esophageal epithelial cells. Reduced Sox2 expression blocks the formation of stratified squamous epithelium.[4] Transcription factor p63, a member of the p53 family, is a potent regulator of the conversion of columnar into stratified squamous epithelium in the esophagus. The esophageal epithelium fails to stratify and remains simple columnar epithelium in p63 mutants. During tracheal separation, the negative regulation of BMP signaling causes persistent activation of Sox2 with repression of Nkx2.1, resulting in squamous differentiation of basal progenitor cells and eventually complete columnar to squamous epithelium transition. The transition of columnar to squamous epithelium may also represent a process of metaplasia. Interestingly, Barrett's esophagus (BE), a reverse metaplasia of the squamous epithelium lining the distal esophagus into an intestinalized columnar epithelium, can occur secondarily to long-term inflammation and injury caused by gastroesophageal reflux. The molecular mechanisms underlying this reversed metaplasia and the cell origin are still under investigation. Treatment with acidified media and/or bile salts *in vitro* mimicking gastroesophageal reflux or using bile acid reflux mouse models have demonstrated the downregulation of squamous transcription factors (e.g., p63 and Sox2), upregulation of columnar (e.g., Sox9), intestinal (e.g., Cdx1 and Cdx2), and mucin (e.g., Foxa2) associated transcription factors, as well as alterations in various signaling pathways that are involved in the development of BE.[9–11]

In summary, the development of esophagus is a dynamic process. The two major processes, separation of the anterior foregut into the trachea and esophagus, and subsequent development of the esophagus, involve reciprocal interactions between the epithelium and the mesenchyme that are mediated by complex signaling pathways and transcription factors. Identifying and understanding the underlying molecular mechanisms of esophageal development, conversion of simple columnar into stratified squamous epithelium, and reversion of stratified squamous epithelium back to columnar epithelium will promote greater insights into the pathophysiology of esophageal diseases.

The squamous epithelium

The word esophagus is derived from the ancient Greek words "oisein," which means "to carry," and "phagein," which means "to eat." The function of the esophagus is simply to carry food into the stomach. It has no known metabolic, endocrine, or digestive function. As a result, the lining epithelium of the esophagus needs to be such that it can withstand a reasonable degree of mechanical and/or chemical trauma. A simple stratified nonkeratinizing squamous epithelium serves this purpose by providing an excellent protective barrier against the partially modified food stream. The three layers of squamous epithelium have slightly different functions. The stratum corneum, also known as the functional layer, is the most superficial layer that is four to five cell layer thick. It is impervious to any luminal contents. Stratum spinosum beneath the corneum, in contrast, has very prominent desmosomes, and allows active transportation of molecules across the cell junctions. The stratum basalis, also known as the basal layer, is two to three cell layer thick. It is the proliferative zone of the epithelium and compensates for the high turnover of superficial epithelial cells following injury.[12] Interspersed

within the squamous epithelium, primarily in the basal layer, are melanocytes and Merkel cells.

Squamous epithelial injury

Regardless of the nature of the stimulus, squamous epithelial injury manifests in a finite set of responses. In fact, a multitude of stimuli can manifest with similar patterns of injury. These can be broadly categorized into inflammatory, pauci-inflammatory, cytologic changes, and proliferative/regenerative changes.

Inflammatory response

Recruitment of inflammatory cells is often the initial manifestation of injury. In most instances, certain types of stimuli result in a predominantly neutrophil-rich, predominantly eosinophil-rich, or predominantly lymphocyte-rich response. For example, erosive gastroesophageal reflux disease (GERD), infections (especially *Candida* spp. and Herpes simplex virus), and pill esophagitis are associated with marked neutrophilic epithelial injury, erosion, and ulcer formation.[13] Eosinophilic esophagitis, GERD, parasitic infections, Crohn's disease, drug hypersensitivity, hypereosinophilic syndrome, celiac disease, vasculitis, and collagen vascular disorders are commonly associated with increased intraepithelial eosinophils.[14] Lymphocytes tend to be a predominant component of inflammatory cells in chronic GERD, drugs-/medications-related injury, Crohn's disease (especially children), achalasia/motility disorders, autoimmune diseases, immunodeficiency (e.g., HIV and CVID), celiac disease, as well as dermatologic conditions, among others.[15]

Pauci-inflammatory response

In some conditions, such as causative or corrosive injury, the esophageal epithelium undergoes extensive necrosis following direct exposure to acids or alkaline agents. There is very little time for the epithelium to elicit an inflammatory response. Similarly, esophagitis dissecans superficialis or "sloughing esophagitis" is believed to be a manifestation of direct mucosal contact with various types of stimuli, such as drugs/medications (especially bisphosphonates and NSAIDs), hot beverages, and chemical irritants.[16,17] Graft-versus-host disease and CVID are examples of immune-mediated injury where the squamous epithelium shows minimal changes of dyskeratosis or single cell apoptosis, without significant inflammation.

Cytologic changes

Dilatation of intercellular spaces (DIS), or spongiosis, almost always accompanies most forms of epithelial injury. Given that this finding has been observed in up to 30% of asymptomatic patients, as well as in response to several stimuli such as erosive GERD, nonerosive GERD, bile acids, and stress, it has limited specificity.[18] Although the molecular mechanisms of DIS are not entirely clear, based on the impedance and ultrastructural studies, it appears that the degree of DIS is directly proportional to the diminished transepithelial resistance and increased esophageal mucosal permeability.[19]

A less common manifestation of epithelial injury is ballooning change. The squamous epithelial cells appear pale and filled with eosinophilic fluid. This fluid represents plasma proteins that have accumulated within the cytoplasm of the epithelial cells following cellular injury.

Epithelial proliferation/regenerative changes

Basal cell hyperplasia and regenerative epithelial changes occur concurrently with most aforementioned forms of injury. In some cases, epithelial injury results in papillomatosis, hyperkeratosis, or parakeratosis.

Basal cell hyperplasia is characterized by expansion of the basal proliferative zone, papillary height elongation, and increased mitotic activity (typically restricted to the basal cells) It imparts a hyperchromatic appearance to the squamous epithelium.[20] In some patients with chronic reflux disease and eosinophilic esophagitis, the mucosa may show basal cell hyperplasia and marked papillary hyperplasia consistent with papillomatosis.

Esophageal hyperkeratosis is a condition where the squamous epithelium shows a distinct granular layer and overlying acellular keratin. In a prospective analysis of 1845 esophageal biopsies, Taggart *et al.* documented the prevalence rate of hyperkeratosis as 2%.[21] In their cohort consisting of 98 patients, hyperkeratosis was found in two clinical settings: (1) patients with BE and BE-associated adenocarcinoma and (2) those without BE. There was no clinical significance to the finding of hyperkeratosis when it was associated with BE. In contrast, non-BE patients with hyperkeratosis showed multifocal involvement with a predilection to involve the

mid-esophageal region. These patients were either current or former alcohol users. More importantly, the non-BE patients showed a high frequency of concurrent or prior history of esophageal squamous neoplasia (67%) or head and neck squamous lesions (31%). In contrast to hyperkeratosis, parakeratotic squamous epithelium shows epithelial hyperplasia with retention of the nuclei within the stratum corneum layer. There appears to be no clinical significance to this finding.

What do these stimuli have in common? Based on our current understanding of the pathogenesis of epithelial injury, it appears that stimuli that result in recruitment of inflammatory cells (neutrophils, eosinophils, or lymphocytes) share a common cytokine-mediated pathway of pathogenesis. Regardless of whether the stimuli are acid, bile salts, or pancreatic enzymes that lead to recruitment of neutrophils;[22,23] or are allergens that elicit an eosinophil-rich inflammatory response in genetically susceptible individuals;[24] or arise from an immunologic response to an ingested agent that causes lymphocyte recruitment, it appears that all of these stimuli in some way or form compromise the mucosal integrity and cause high transepithelial permeability.[25,26] This results in release of cytokines and growth factors that ultimately leads to recruitment of inflammatory cells, DIS, and basal cell hyperplasia.

In summary, the human esophagus is lined by stratified squamous epithelium to serve as a protective barrier from potentially harmful luminal agents. When exposed to an injurious agent/stimulus, the most common reactions to injury include recruitment of inflammatory cells, DIS, and a rapid attempt to regenerate the injured squamous epithelium, which manifests as basal cell hyperplasia. All of these reactions can result from multiple different types of stimuli; however, they appear to share a common pathway of cytokine-mediated injury.

The LP and MM

The normal LP contains loose collagen, blood vessels, lymphatic channels, and lymphocytes. In contrast to normal squamous-lined mucosa in which the LP forms a distinct and compact layer, in esophagi of patients with BE, the LP contains glandular epithelium similar to the other columnar-lined segments of the GI tract. The most striking changes in the LP are related to the MM, which in patients with BE undergo duplication, fragmentation, and expansion. Below we discuss the characteristics and prevalence rate of MM alterations, its pathogenesis, histologic properties, and finally, the clinical implications of this phenomenon.

MM alterations

The original description of MM alterations in BE was by Rubio *et al.* in 1988. In an evaluation of 32 esophageal resections performed for BE-associated adenocarcinoma, the authors found thickening of the MM, with extension of smooth muscle fibers into the LP in 26 of 32 (81%) cases.[27] However, the first study to describe duplication of the MM in BE was by Takubo *et al.* Esophagectomies from eight patients with BE were compared to 352 esophagectomies from patients without BE. Duplication of the MM was observed in 87% of BE patients, but in none of the controls.[28] This study showed that in BE, a new layer of MM develops more superficial (luminal) to the original (deep) layer of MM native to the squamous-lined esophagus. The superficial (newly developed) and deep layers of MM ultimately converge into one layer at the neo squamocolumnar junction, but distally at the level of the distal GEJ, the superficial layer becomes attenuated and is replaced by fibrous tissue. A study by Abraham *et al.* showed similar findings.[29] In the study, 46 of 50 (92%) BE resections demonstrated "duplicated" MM, which involved from 5% to greater than 90% of the BE segment. However, none of the 20 resected squamous cell carcinomas showed changes in the MM. Interestingly, in 5 (10%) cases, the MM was focally divided into three distinct layers. In a subsequent study by Lewis *et al.*, the authors analyzed the MM in endoscopic mucosal resection (EMR) specimens and found that MM duplication was present in 73 of 111 (66%) EMR specimens.[30]

Given that duplication of the MM in BE is a common phenomenon, the implications with regard to staging carcinomas and the risk of metastasis are of prime importance. For instance, one important question is whether carcinomas that infiltrate into, or through, the newly developed (superficial) MM behave similarly to true submucosally invasive cancers, or do they behave more similar to "intramucosal" (IMC) cancers? Hahn *et al.* evaluated the vascular and lymphatic properties of the mucosa and SM in BE patients with a duplicated MM in an effort to determine the potential impact of this

phenomenon on staging superficial carcinomas.[31] In a cohort of esophagogastrectomy specimens from 30 patients with BE-associated adenocarcinoma ($n = 6$), IMC adenocarcinoma ($n = 26$), or high-grade dysplasia ($n = 2$), the density of $CD31^+$ blood and lymphatic vessels in the superficial ($n = 37$) and deep LP ($n = 38$) was found to be significantly lower compared to the LP of normal squamous-lined esophagus ($n = 68$). However, the total number of blood and lymphatic vessels in the combined layers was statistically similar to the LP of squamous-lined esophagus. The density of $CD31^+$ blood and lymphatic vessels in the SM of BE was not significantly different from the SM of squamous-lined esophagus. These findings suggested that carcinomas that invade through the superficial MM into the deep LP may behave biologically similar to "intramucosal" rather than "submucosal" cancers, with regard to the risk of lymphatic or blood vessel invasion and metastasis.

Implications for staging early adenocarcinomas in BE

The presence of a duplicated MM in BE has led to challenges with regard to staging superficially invasive cancers. Currently, the American Joint Commission on Cancer/Union for International Cancer Control classifies neoplastic glands that invade into the superficial LP, deep LP (space between superficial and deep of MM) and the deep MM as pT1a.[32] Invasion beyond the deep MM and into the true SM is categorized as pT1b. In fact, the risk of lymph node (LN) metastasis has been shown to correlate with depth of invasion. In a series of 272 endoscopic resections, Vieth *et al.*[33] classified depth of invasion into four levels: m1-invasion into superficial LP, m2-invasion into superficial (newly formed) MM, m3-invasion into the space between the two layers of MM, and m4-invasion into deep MM. This study showed that the incidence of lymphatic invasion is very low in adenocarcinomas that invade the m1 (0.8%), m2, or m3 (0%) levels, and progressively increases in cancers with level m4 (2.8%) and submucosal invasion (13–20%).[33] Thus, IMC adenocarcinoma has a much lower risk of LN metastasis (0–3%) compared to submucosally invasive adenocarcinoma (8–36%).[33] In another study of 99 BE-associated pT1 cancers, Estrella *et al.* found LN metastasis in one (3%) patient with tumor that invaded into the LP/inner MM, zero patients with tumor that invaded the space between the superficial and deep LP, and 10 (33%) patients with tumor that invaded the true SM.[34]

In summary, most patients with BE develop either a partial, or complete, duplication of the MM, which is situated in the original LP above the original (deep) MM of the native squamous-lined esophagus. Although MM alterations result in the formation of a "superficial" and "deep" LP, the properties of the combined superficial and deep LP are similar to the original LP. The rate of LN metastasis (and recurrence) in superficially invasive adenocarcinomas that infiltrate into the superficial or deep LP is similar, but significantly different compared to adenocarcinomas with true submucosal invasion. Therefore, it is important to recognize appropriate histologic landmarks and distinguish "mucosal" from true "submucosal" invasion when staging superficially invasive esophageal adenocarcinomas.

The submucosa

The SM of the esophagus provides a flexible matrix, which serves as a cushion between mucosa and MP during peristalsis. It is also the regional routing center for blood and lymphatic flows. Histologically, the SM is made of loosely arranged collagen, elastic fibers, and adipose tissue with embedded relatively large caliber arterioles, venules, and lymphatic vessels. Neural structures and variable amount of scattered inflammatory cells are also components of the SM.

A unique structure in the esophageal SM is the submucosal mucus gland, which is thought to be the result of invagination of the surface epithelium during embryonic development or continuation of the minor salivary glands of the oropharynx. The presence of submucosal glands or their ducts in biopsies is indicative of an esophageal location, which may facilitate a diagnosis of BE by confirming the esophageal origin of the sampled specialized columnar epithelium.[35]

A rich lymphatic network is present in the LP and is further concentrated in the SM. Several studies had suggested that lymphatics within the SM drains longitudinally along the submucosal plexuses up to its proximal ends (recurrent laryngeal nodes/ supraclavicular node) or down to its distal ends (paracardial nodes/celiac nodes),[36] bypassing the network in MP/adventitia and regional LNs. Direct drainage

into the thoracic duct has also been documented in autopsy studies.[37,38] The exact drainage pathways may be highly variable among individuals[39–41] and may explain "skip metastasis" as reported in some patients with thoracic esophageal carcinoma.

While there is no anatomic landmark to divide the layers within the SM, increasing clinical interest in excising carcinomas with superficial submucosal invasion using endoscopic approach[42] demands a unified method of documenting the depth of cancer invasion. The commonly used methods, the Pragmatic classification (subdivision of the SM into three equal layers) and the Paris classification for stomach (submucosal invasion $\leq$500 μm as SM1, 500–1000 μm as SM2, and $>$ 1000μm as SM3),[43,44] both suffer from inconsistency created by observer subjectivity and processing artifact. While most of the studies on tumor depth and risk of LN metastasis used surgical resection specimens and the pragmatic approach, due to incompleteness of submucosal layer in endoscopic resection specimens, the Paris classification may become the only solution. As a crucial buffering layer between the mucosa and the more rigid MP, the submucosal response after mucosal injury plays an important role in stricture formation after EMR or submucosal dissection. In animal models, starting from the second day after a procedure, prominent inflammatory infiltrates are seen in the SM with a significant neutrophilic component. In the next 2 weeks, inflammation decreases and angiogenesis increases. By around 28 days after the procedure, in addition to dense fibrosis in the SM, the muscle layer also shows significant atrophy and fibrosis, which further reduces contractibility and flexibility of the esophageal wall.[45,46]

The muscularis propria

The esophageal MP, through most of its length, like that in the rest of the gut, has two layers, an inner layer of circular smooth muscle and an outer layer of longitudinal muscle. In general, the inner layer is thicker than the outer. Between these layers it the myenteric nerve plexus. In the upper third, there is a mixture of skeletal and smooth muscle, with gradual loss of the skeletal muscle as the thoracic part of the esophagus is reached.

Specific diseases that target the MP

Atrophy and fibrosis of the MP was found in 94% of autopsies of patients diagnosed with scleroderma during life. Atrophy of the circular layer is dramatically more severe than that of the longitudinal layer.

Achalasia is associated with inflammation of the myenteric plexus of the MP. End-stage achalasia is characterized by the absence of ganglion cells and fibrosis of the nerves of the myenteric plexus. Prominent hypertrophy of the circular layer of the MP is also characteristic.

Leiomyoma constitutes approximately 60–70% of all esophageal mesenchymal tumors. It has low cellularity, no atypia, and no mitoses.

Gastrointestinal stromal tumors (GIST) of the esophagus constitute less than 1% of all GISTs. Esophageal GISTs are overwhelmingly c-KIT-positive by immunohistochemistry. They are also more aggressive than gastric GISTs. The criteria used for the assessment of the risk of malignant behavior are the same as for the jejunum/ileum GISTs.

Distinguishing between duplicated MM and MP in endoscopic mucosal resections

The deep MM is contiguous with the original MM of the squamous esophagus and continues caudally merging with the MM of the stomach. The LP of the squamous esophagus is contiguous with the space between the duplicated MM. Below the deep MM is the SM. Invasion of adenocarcinoma into the duplicated MM space is interpreted as IMC carcinoma. Because of its patchiness, duplicated MM is seen only in a half to two-thirds of the EMR specimens.

It may be difficult to decide in the EMR sections whether adenocarcinoma invading beyond the only layer of MM is IMC or submucosal. When a second muscle layer is present at the deep margin, it may be challenging to differentiate the deep MM from the MP. Yet, the distinction is important, as invasion into the duplicated MM space can be treated endoscopically, while submucosal invasion is treated with esophagectomy. In addition, presence of MP is a worrisome sign of an increased risk of perforation.

Recognition of the SM will allow distinction between the deep MM and the MP, because it is positioned underneath the deep MM and above the MP. Distinctive features of the SM are salivary-type glands, adipose tissue, and large-caliber muscular vessels. The vessels in the SM are larger, thicker, and

more tortuous and clustered than the vessels in the superficial LP or the duplicated MM space. Using the presence of the salivary-type glands, adipose tissue, and large-caliber muscular vessels, Kaye *et al.* have recently demonstrated an excellent agreement in recognition of the SM in the EMR specimens, with kappa values between 0.69 and 0.96.[47]

The nerve supply of the esophagus

The esophagus receives predominantly parasympathetic nerve supply from the vagus, and sympathetic nerve fibers form the cervical and paravertebral chains.[48]

The intrinsic nerve supply is composed of two nerve plexuses (ganglia, axons, nerve fibers): Auerbach's myenteric plexus and Meissner's submucosal plexus. Meissner's submucosal plexus has a (1) a superficial component, close to the MM, (2) Henle's plexus—the deep component adjacent to the circular layer of MP, and (3) a less well-defined intermediate plexus.[48]

The history of nomenclature of the enteric plexuses includes the following.[48] Henle in 1871 described the plexus myentericus externus (between the longitudinal and circular muscle layer) and plexus myentericus internus (on the outer surface of the MM). The plexus myentericus externus of Henle corresponds to the myenteric, not the submucosal, plexus. The Russian histologist Schabadash was the first to describe two different submucosal plexus types, an outer and an inner one. However, because he misunderstood Henle's text, he called the outer submucosal plexus (close to the surface of the circular muscle) and the inner submucosal plexus: "plexus externus Henle" and "plexus internus submucosus Meissner," respectively.

The interstitial cells of Cajal (ICCs) are present in the SM, intermuscular, and intramuscular layers of the esophageal wall.[48] ICCs are present in the mid-esophagus associated with smooth and striated muscle and in the distal esophagus associated with smooth muscle.[49] ICCs are concentrated in the smooth muscle of the esophagus and within the LES. Unlike in the small and large bowel, ICCs do not aggregate around the myenteric plexus or at the submucosal border, as they do in the intestines.[50,51] The ICCs play an important role in gut motility and serve as pace makers of motility. Frequent gap junctions between the ICCs are described in ultrastructural studies and form a network throughout the bowel wall. ICCs are present in close apposition to nerve varicosities and are richly innervated by the local nerve fibers.[52] Evidence for the role of ICCs in gut motility and internal pace making activity has accumulated since their discovery.[53–55] The pacemaker activity is most concentrated in the ICCs in small intestine and stomach.[56,57] ICC within the esophageal muscle layers show little evidence of the slow depolarization wave production characteristic of pacemaker cells; thus not all ICCs are involved in pace-making activities.

When ICCs are absent[58,59] or knocked out in a mouse model,[60] pacemaker activity is lost. It appears diseases in which ICCs are implicated relate to decreased number of ICCs and developmental delay. It remains unclear whether these abnormalities represent primary or secondary events affecting the ICCs.

Classification of neuromuscular pathology of the GI tract can be challenging due to the large number of entities involved, potential overlap, and the multiple ways in which they can be catalogued. The London Classification offers a structured classification of histologic phenotypes based on robust contemporary histopathologic criteria with correlation between histopathological phenotypes and entities in clinical practice.[61]

Vascular and lymphatic supplies of the esophagus

Lymphatic supply within the esophagus begins in the LP and travels in the LP and SM until larger lymphatics terminate either directly in the thoracic duct, especially from the right and dorsal sides of the esophagus, or in the remaining esophagus often being relayed through LNs. The larger lymphatics penetrate the wall of the esophagus and each of these may drain up to about 40 mm of esophageal SM.[38] The vagaries of drainage can be seen by studying the sites of nodal metastases from small carcinomas and the sites to which they preferentially drain.[62] However, these do not get to the issue of why there are so many lymphatics in the esophagus, especially when it is assumed there is no absorption.

Why so many lymphatics in the esophagus when it is assumed there is no absorption?

This is an interesting question. Absorption can certainly occur through the skin and squamous

mucosa of the mouth, so there is no reason why a small degree of absorption should not take place in the esophagus, albeit being limited by contact time.

The three main areas containing lymphatics are in the LP including the MM, the SM between the MM, and MP those in the adventitia and beyond. The corollary is whether the density of lymphatics is the same throughout the esophagus and whether there is any change with age, and therefore growth. Defining the lamina in intrauterine life is problematic as there is no MM in the upper part of the esophagus, so that the LP and SM are in continuity, and even in adults this remains thin, but is present. Further, using both CD31 and D240 immunohistochemistry, there appears to be an increase in the density of lymphatics from proximal to distal in both intrauterine life and in adults.

Are there diseases that lead to vascular and lymphatic alterations?

Congenital lymphangiectasia is incredibly rare[63] and Milroy's disease (congenital lymphangiectasia) is not described as affecting the esophagus. Dilated lymphatic s can be seen in patients with carcinomas obstructing lymphatics. However, a variety of vascular disease can affect the esophagus, by far the most significant clinically are esophageal varices in patient with portal hypertension. Many insults and diseases can alter vascular and lymphatic supply, including

- Acute esophageal necrosis (Gurvits syndrome, black esophagus, acute necrotizing esophagitis, esophageal infarction), vascular/hypoperfusion, shock, atheroma, vasoconstricting agents (cocaine), necrotizing arteritis.
- Chemical injury, e.g., corrosives, acid, alcohol, medications.
- Metabolic abnormalities, e.g., hyperglycemia, uremia, sepsis, lactic acidosis, anemia, hypoxia, hypoproteinosis.
- Infections, e.g., CMV, herpes, mycotic.
- Mechanical injury, mostly iatrogenic, e.g., surgical manipulation, trauma from nasogastric tubes.
- Comorbidities, e.g., peptic ulcers, renal insufficiency, coronary artery disease/congestive heart disease/CHF, cirrhosis/metabolic syndrome, pulmonary disease, immune compromise diseases.

The inlet patch

The "inlet patch" refers to a discrete focus (or foci) of gastric-type mucosa in the cervical esophagus. The term was coined by Jabbari *et al.* in their 1985 prospective endoscopy study, which encompasses most of its key clinicopathologic features.[64] The inlet patch had been referred to previously as ectopic or heterotopic gastric mucosa of the upper (proximal, cervical) esophagus, mainly in the setting of case reports of symptomatic patients. Jabbari *et al.* found an endoscopic prevalence of 3.8% (8M:8W) in 420 consecutive upper endoscopies.[64] All lesions were located $\leq$3 cm from the upper esophageal sphincter, ranged in size from 2 mm to circumferential, and were single (88%) or paired (12%). One patient, who happened to have the largest inlet patch in the series, had throat discomfort, which was relieved by an H_2 blocker. Endoscopic mucosal biopsy material demonstrated corpus or cardiac-type mucosa; no patient had intestinal metaplasia of the inlet patch. Inlet patches produced acid on pentagastrin stimulation. One patient (6.3%) had concurrent BE.

Inlet patches appear to represent developmental residua—a conclusion based on detailed morphologic analysis of human embryos and its frequent detection in pediatric patients (the greatest reported inlet patch prevalence is from a pediatric autopsy study). The earliest recognizable esophageal lining is a stratified columnar epithelium (i.e., at the 3 mm crown rump-length stage).[65] Perhaps inlet patches are residuals of this columnar lining that have undergone maturation to gastric mucosae.

The reported prevalence of the inlet patch (0.1–21%)[66,67] has varied widely depending on who looks, how they look, and how hard they look and does not appear to vary based on the nature of the population studied. In a recent prospective endoscopy study, Peitz *et al.* reported a prevalence of 14.5% (54/372); when this same group looked back at nearly 10,000 of their prior upper endoscopies, it had been documented in only 0.5%.[68] A few prospective studies have compared the prevalence in the operator aware (i.e., endoscopist with knowledge that the purpose of the study is to determine inlet patch prevalence) versus operator unaware settings, with the prevalence typically six times higher in the former.[69] In some studies, narrow band imaging or high-definition white light endoscopy have been shown to increase the detection rate. The

vast majority of studies have recruited patients presenting for upper endoscopy, though Govani *et al.* reported a prevalence of 6.9% in volunteers.[70]

Although most patients do not have symptoms referable to their inlet patch, the most frequently attributed are laryngopharyngeal, including globus, cough, and laryngospasm. Rarely, large inlet patches have been reported to cause strictures, rings, webs, bleeding, ulceration, or perforation. In patients with attributable symptoms, inlet patches can be endoscopically ablated.[71] Helicobacter is variably detected in the inlet patches of patients in whom the stomach is infected. A half dozen studies have reported a positive association between the presence of an inlet patch and concurrent BE, but just as many studies have failed to demonstrate an association. It is possible that endoscopists may have looked harder (even subconsciously) for inlet patches in the setting of Barrett's mucosa. Intestinal metaplasia is uncommonly seen in biopsy material from inlet patches (3% of 2000 cases across a couple dozen studies), and upper esophageal adenocarcinoma, possibly arising in inlet patches, is exceptional, with only 58 previously reported cases. As such, inlet patches do not routinely need to be biopsied because of the possibility of dysplasia or carcinoma.[72]

The submucosal glands and their ducts, and the cardiac glands

Function and microanatomy of submucosal glands and ducts

Submucosal glands and ducts play an important role in maintaining the seromucinous pre-epithelial barrier of the squamous mucosa.[73] They secrete biologically active peptides, including trefoil factor family 3, epidermal growth factor, transforming growth factor-alpha, and prostaglandin E2 to maintain the integrity of the squamous mucosa.[74] Submucosal glands also secrete a variety of defensive cell products; neutral and sialylated mucins prevent viruses and bacteria from infiltrating the underlying mucosa, lysozymes are bactericidal, and pepsinogen is activated to pepsin, which contributes to proteolysis.

Submucosal glands and their ducts are arranged in rows parallel to the long axis of the esophagus. Aggregates of two to five lobules drain into a common duct that penetrates the squamous epithelium and extends to the surface. These ducts contain two cell layers. An inner layer of short columnar epithelial cells is supported by an outer layer of smaller cuboidal cells; both are surrounded by a cuff of lymphocyte-rich mononuclear cell inflammation. As the ducts extend to the luminal surface, the flattened cuboidal epithelium gradually transitions to a stratified squamous epithelium subjacent to short columnar cells that line the duct lumen.

Submucosal glands are most numerous in the proximal esophagus, although their presence in the distal esophagus represents a helpful histologic landmark that defines the extent of the tubular esophagus. Submucosal glands consist of acini invested in a peripheral rim of myoepithelial cells; acini contain variable numbers of mucous cells, serous cells, and oncocytic cells. Mucous cells are more numerous and generally predominate in lobules at all levels in the esophagus. They contain sulphomucins that impart a faintly basophilic hue to their cytoplasm and they show strong staining for Alcian blue. Serous cells contain deeply basophilic, granular cytoplasm and peripherally arranged, small, round nuclei; they may be absent from some submucosal glands. Oncocytic cells are cuboidal with abundant, densely eosinophilic cytoplasm and uniform, round nuclei with conspicuous nucleoli.

Function and microanatomy of cardiac-type glands in the esophagus

Cardiac-type glands are normally present in the esophagus where they function to lubricate and protect the mucosa; loss of cardiac-type glands is associated with GERD.[75] Hanada *et al.* performed endoscopic examinations on 2656 patients in search of cardiac-type glands on the proximal side of the GEJ. They identified esophageal cardiac-type glands in 355 (13%) patients. Cardiac-type glands were patchy in 9.7% patients, but appeared as multiple foci over $<50\%$ and $>50\%$ of the esophageal circumference in 1.8% and 1.9% of patients, respectively. Cardiac-type glands were more common among women and their presence was inversely associated with GERD.[76]

Lobules of cardiac-type glands are commonly present in the mucosae of the proximal and distal esophagus, where they appear as white or yellow nodules and plaques.[76] These lobules consist of small aggregates (<10) of glands invested in LP that contains plasma cells and lymphocytes. Glands are lined by columnar to short cuboidal cells with

basally located nuclei and faintly eosinophilic mucinous cytoplasm. These cardiac-type glands are morphologically indistinguishable from cardiac-type glands in the proximal stomach.

Diseases of submucosal glands and their ducts, and cardiac glands

Submucosal glands, ducts, and cardiac glands produce mucins and biologically active peptides that lubricate the esophageal mucosa and protect it from direct luminal injury and pathogens. Inflammatory disorders that involve these structures may pose problems for pathologists who encounter them in biopsy or resection material, but clinically significant diseases affecting these structures are uncommon. Radiation-induced atrophy of glands may cause diagnostic challenges for pathologists in some cases, although their benign nature can usually be discerned owing to the lobular arrangement of glandular elements, many of which show variable dilation and attenuated epithelium. Although radiation may induce single cell necrosis in benign glands, nuclear enlargement is generally accompanied by concomitant increases in cytoplasmic volume and an absence of mitotic activity.

Intramural diverticulosis (pseudodiverticulosis) is a clinically asymptomatic disorder characterized by diffusely dilated submucosal glands and ducts throughout the esophagus. Most cases occur in patients with underlying esophageal motility disorders or strictures. Presumably, increased intraluminal pressures result in herniation of submucosal glands and their supportive tissue into the MP. Dilated excretory ducts and glands are typically associated with variable amounts of inflammation and fibrosis, reminiscent of Rokitansky–Aschoff sinuses in the gallbladder. Isolated cysts derived from esophageal ducts can also occur, resulting in an endoscopically apparent bulge or nodule that usually spans less than 1 cm. Cysts contain mucin and may display papillary intraluminal folds, but lack cytologic atypia. Most examples are encountered among patients with GERD.[77]

Glandular elements in the tubular esophagus may give rise to esophageal adenocarcinomas that develop in the upper and mid-esophagus unrelated to columnar-lined esophagus. Nie *et al.* identified three examples of an entity they classified as esophageal submucosal gland duct adenoma.[77] These lesions consisted of multiple cysts lined by flat, undulating, or slightly papillary epithelium. All three cases featured two layers of epithelial cells with luminal ductal cells and basal cuboidal cells. The proliferative indices of all three cases were $< 1\%$ and all showed only minimal to mild cytologic abnormalities. It is not clear whether these lesions represented neoplasms or exuberant hyperplasia.

There are a few well-documented case reports of adenocarcinoma derived from submucosal glands and ducts, most of which have been reported in the Japanese literature. Unlike adenocarcinomas associated with BE and squamous cell carcinoma, those derived from esophageal glands seem to affect men and women equally and occur in older adults. Early lesions may appear as a nodule, ulcer, or depressed area, often occurring in the upper or mid-esophagus. Most tumors resemble carcinomas that develop in the salivary glands with mucoepidermoid carcinoma being the most common variant.[78] Of note, most historical examples of esophageal adenoid cystic carcinoma represent squamous cell carcinomas with prominent basaloid features, and many reported cases of mucoepidermoid carcinoma show high-grade cytologic features that warrant classification as adenosquamous carcinoma.

In summary, esophageal cardiac glands, submucosal glands, and their ducts are normally present throughout the esophagus, and are more numerous in the proximal and distal esophagus. Their primary function appears to be maintenance of mucosal integrity and lubrication of the esophageal mucosa. Although these structures may be subject to inflammatory or metaplastic alterations that pose diagnostic challenges for surgical pathologists, clinically significant diseases are exceedingly uncommon. Esophageal adenocarcinomas may be derived from esophageal glands in some cases, although well-documented cases of cancers derived from these structures are uncommon.

The GEJ and the LES

The GEJ has different definitions depending on the discipline that studies it. Anatomic, physiologic, histologic, and endoscopic definitions of the GEJ exist.

In healthy individuals, the GEJ is anatomically defined as the transition of the esophagus to the gastric cardia, which also corresponds to angle of His,[79] or where the esophagus and stomach meet (Z line). Histologically, it is defined as the junction of squamous and columnar mucosa.[79–81] Several different

endoscopic criteria for defining the GEJ exist, but the most commonly used and reproducible one is the "proximal margin of the gastric folds," although "distal end of esophageal palisading longitudinal vessels" is also being used.[79,82,83] In addition, AJCC 2010 defines the GEJ as "the junction of the tubular esophagus and the stomach, irrespective of the type of epithelial lining of the esophagus."[84] However, all of these definitions may not correspond to the exact same area. Identifying the correct location of the GEJ has several important clinical implications, including diagnosis and endoscopic grading of BE,[79,84,85] staging of GEJ and stomach cancers,[84,86] and surgical classification and management of GEJ tumors.[87,88]

From a physiologic perspective, the GEJ is generally defined as the manometric high pressure zone at the lower esophagus, which separates the negative pressure of the thoracic esophagus from the positive pressure of the stomach.[89] This area corresponds to the LES. The LES is not a true anatomic sphincter and this is a topic of continuous debate.[79,81,89,90] Currently it is believed that the LES consists of several different components, to include the gastric clasp muscle (located at the lesser curvature of the stomach), gastric sling muscle (located at the cardia), longitudinal outer smooth muscle, and the crural diaphragm that serves as an antireflux barrier.[81,89,90] The phrenoesophageal ligament attaches the lower esophagus to the diaphragm and brings the distal esophagus back to neutral position following peristalsis.[81] Proper functions of these structures play an important role in swallowing and reflux/antireflux mechanisms.

Concluding comments

To summarize, as can be seen from this detailed discussion, the esophageal wall, from mucosa through MP, is beautifully designed to fulfill its limited function as a conduit, bringing materials from the mouth and oropharynx to the stomach. Each of its layers and special structures, including blood and lymphatic vessels and nerves, respond to a variety of insults and injuries in remarkable ways, many of which have been detailed above. It is remarkable that a part of the gut, the esophagus—so short and so narrow—has so many diseases intrinsic to it.

Competing interests

The authors declare no competing interests.

References

1. Zhang, Y., M. Jiang, E. Kim, *et al.* 2017. Development and stem cells of the esophagus. *Semin. Cell Dev. Biol.* **66:** 25–35.
2. Jacobs, I.J., W.Y. Ku & J. Que. 2012. Genetic and cellular mechanisms regulating anterior foregut and esophageal development. *Dev. Biol.* **369:** 54–64.
3. Perin, S., C.J. McCann, O. Borrelli, *et al.* 2017. Update on foregut molecular embryology and role of regenerative medicine therapies. *Front. Pediatr.* **5:** 91.
4. Que, J., X. Luo, R.J. Schwartz & B.L. Hogan. 2007. Multiple dose-dependent roles for Sox2 in the patterning and differentiation of anterior foregut endoderm. *Development* **134:** 2521–2531.
5. Billmyre, K.K., M. Hutson & J. Klingensmith. 2015. One shall become two: separation of the esophagus and trachea from the common foregut tube. *Dev. Dyn.* **244:** 277–288.
6. Domyan, E.T., E. Ferretti, K. Throckmorton, *et al.* 2011. Signaling through BMP receptors promotes respiratory identity in the foregut via repression of Sox2. *Development* **138:** 971–981.
7. Que, J. 2015. The initial establishment and epithelial morphogenesis of the esophagus: a new model of tracheal-esophageal separation and transition of simple columnar into stratified squamous epithelium in the developing esophagus. *Wiley Interdiscip. Rev. Dev. Biol.* **4:** 419–430.
8. Chihara, D., A.I. Romer, C.F. Bentzinger, *et al.* 2015. PAX7 is required for patterning the esophageal musculature. *Skelet. Muscle* **5:** 39.
9. Wang, D.H. & R.F. Souza. 2016. Transcommitment: paving the way to Barrett's metaplasia. *Adv. Exp. Med. Biol.* **908:** 183–212.
10. Wang, D.H. 2017. The esophageal squamous epithelial cell—still a reasonable candidate for the Barrett's esophagus cell of origin? *Cell Mol. Gastroenterol. Hepatol.* **4:** 157–160.
11. Jiang, M., H. Li, Y. Zhang, *et al.* 2017. Transitional basal cells at the squamous–columnar junction generate Barrett's oesophagus. *Nature* **550:** 529–533.
12. El-Zimaity, H. & R. Riddell. 2012. Esophagus. In *Histology for Pathologists.* 4th ed: S.E. Mills, Ed.: 605–632. Philadelphia, PA: Lippincott Williams & Wilkins.
13. Grin, A. & C.J. Streutker. 2015. Esophagitis: old histologic concepts and new thoughts. *Arch. Pathol. Lab. Med.* **139:** 723–729.
14. Fahey, L.M. & C.A. Liacouras. 2017. Eosinophilic gastrointestinal disorders. *Pediatr. Clin. North Am.* **64:** 475–485.
15. Nguyen, A.D. & K.B. Dunbar. 2017. How to approach lymphocytic esophagitis. *Curr. Gastroenterol. Rep.* **19:** 24.
16. Carmack, S.W., R. Vemulapalli, S.J. Spechler, *et al.* 2009. Esophagitis dissecans superficialis ("sloughing esophagitis"): a clinicopathologic study of 12 cases. *Am. J. Surg. Pathol.* **33:** 1789–1794.
17. Purdy, J.K., H.D. Appelman & B.J. McKenna. 2012. Sloughing esophagitis is associated with chronic debilitation and medications that injure the esophageal mucosa. *Mod. Pathol.* **25:** 767–775.

18. van Malenstein, H., R. Farré & D. Sifrim. 2008. Esophageal dilated intercellular spaces (DIS) and nonerosive reflux disease. *Am. J. Gastroenterol.* **103:** 1021–1028.
19. Tobey, N.A., J.L. Carson, R.A. Alkiek, *et al.* 1996. Dilated intercellular spaces: a morphological feature of acid reflux–damaged human esophageal epithelium. *Gastroenterology* **111:** 1200–1205.
20. Allende, D.S. & L.M. Yerian. 2009. Diagnosing gastroesophageal reflux disease: the pathologist's perspective. *Adv. Anat. Pathol.* **16:** 161–165.
21. Taggart, M.W., A. Rashid, W.A. Ross, *et al.* 2013. Oesophageal hyperkeratosis: clinicopathological associations. *Histopathology* **63:** 463–473.
22. Weijenborg, P.W., A.J.P.M. Smout, C. Verseijden, *et al.* 2014. Hypersensitivity to acid is associated with impaired esophageal mucosal integrity in patients with gastroesophageal reflux disease with and without esophagitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* **307:** G323–G329.
23. Altomare, A., M.P.L. Guarino, S. Cocca, *et al.* 2013. Gastroesophageal reflux disease: update on inflammation and symptom perception. *World J. Gastroenterol.* **19:** 6523–6528.
24. Blanchard, C. & M.E. Rothenberg. 2008. Basic pathogenesis of eosinophilic esophagitis. *Gastrointest. Endosc. Clin. N. Am.* **18:** 133–143.
25. Blevins, C.H., P.G. Iyer, M.F. Vela, *et al.* 2017. The esophageal epithelial barrier in health and disease. *Clin. Gastroenterol. Hepatol.* **17:** 30742–30745.
26. Orlando, R.C. 2010. The integrity of the esophageal mucosa. Balance between offensive and defensive mechanisms. *Best Pract. Res. Clin. Gastroenterol.* **24:** 873–882.
27. Rubio, C.A. & R. Riddell. 1988. Musculo-fibrous anomaly in Barrett's mucosa with dysplasia. *Am. J. Surg. Pathol.* **12:** 885–889.
28. Takubo, K., K. Sasajima, K. Yamashita, *et al.* 1991. Double muscularis mucosae in Barrett's esophagus. *Hum. Pathol.* **22:** 1158–1161.
29. Abraham, S.C., A.M. Krasinskas, A.M. Correa, *et al.* 2007. Duplication of the muscularis mucosae in Barrett esophagus: an underrecognized feature and its implication for staging of adenocarcinoma. *Am. J. Surg. Pathol.* **31:** 1719–1725.
30. Lewis, J.T., K.K. Wang & S.C. Abraham. 2008. Muscularis mucosae duplication and the musculo-fibrous anomaly in endoscopic mucosal resections for Barrett esophagus: implications for staging of adenocarcinoma. *Am. J. Surg. Pathol.* **32:** 566–571.
31. Hahn, H.P., A. Shahsafaei & R.D. Odze. 2008. Vascular and lymphatic properties of the superficial and deep lamina propria in Barrett esophagus. *Am. J. Surg. Pathol.* **32:** 1454–1461.
32. Rice, T.W., D.P. Kelsen, E.H. Blackstone, *et al.* 2017. Esophagus and esophagogastric junction. In *AJCC Cancer Staging Manual.* 8th ed. M.B. Amin, Ed.: 185–202. New York: Springer.
33. Vieth, M. & M. Stolte. 2005. Pathology of early upper GI cancers. *Best Pract. Res. Clin. Gastroenterol.* **19:** 857–869.
34. Estrella, J.S., W.L. Hofstetter, A.M. Correa, *et al.* 2011. Duplicated muscularis mucosae invasion has similar risk of lymph node metastasis and recurrence-free survival as intramucosal esophageal adenocarcinoma. *Am. J. Surg. Pathol.* **35:** 1045–1053.
35. Naini, B.V., R.F. Souza & R.D. Odze. 2016. Barrett's esophagus: a comprehensive and contemporary review for pathologists. *Am. J. Surg. Pathol.* **40:** 45–66.
36. Tachimori, Y. 2017. Pattern of lymph node metastases of squamous cell esophageal cancer based on the anatomical lymphatic drainage system: efficacy of lymph node dissection according to tumor location. *J. Thorac. Dis.* **9**(Suppl. 8): S730.
37. Murakami, G., I. Sato, K. Shimada, *et al.* 1994. Direct lymphatic drainage from the esophagus into the thoracic duct. *Surg. Radiol. Anat.* **16:** 399–407.
38. Kuge, K., G. Murakami, S. Mizobuchi, *et al.* 2003. Submucosal territory of the direct lymphatic drainage system to the thoracic duct in the human esophagus. *J. Thorac. Cardiovasc. Surg.* **125:** 1343–1349.
39. Saito, H., T. Sato & M. Miyazaki. 2007. Extramural lymphatic drainage from the thoracic esophagus based on minute cadaveric dissections: fundamentals for the sentinel node navigation surgery for the thoracic esophageal cancers. *Surg. Radiol. Anat.* **29:** 531–542.
40. Riquet, M., F. Le Pimpec Barthes, R. Souilamas & G. Hidden. 2002. Thoracic duct tributaries from intrathoracic organs. *Ann. Thorac. Surg.* **73:** 892–898.
41. Rice, T.W., M.P. Bronner. 2011. The esophageal wall. *Thorac. Surg. Clin.* **21:** 299–305.
42. Bhatt, A., S. Abe, A. Kumaravel, *et al.* 2015. Indications and techniques for endoscopic submucosal dissection. *Am. J. Gastroenterol.* **110:** 784–791.
43. Anonymous. 2003. The Paris endoscopic classification of superficial neoplastic lesions: esophagus, stomach, and colon: November 30 to December 1, 2002. *Gastrointest. Endosc.* **58**(6 Suppl.): S3–S43.
44. Endoscopic Classification Review Group. 2005. Update on the Paris classification of superficial neoplastic lesions in the digestive tract. *Endoscopy* **37:** 570–578.
45. Honda, M., T. Nakamura, Y. Hori, *et al.* 2010. Process of healing of mucosal defects in the esophagus after endoscopic mucosal resection: histological evaluation in a dog model. *Endoscopy* **42:** 1092–1095.
46. Nonaka, K., M. Miyazawa, S. Ban, *et al.* 2013. Different healing process of esophageal large mucosal defects by endoscopic mucosal dissection between with and without steroid injection in an animal model. *BMC Gastroenterol.* **13:** 72.
47. Kaye, P.V., M. O'Donovan, N. Mapstone, *et al.* 2015. Pathologists are able to reliably differentiate the lamina propria associated with Barrett's musculo-fibrous anomaly from submucosa in oesophageal endoscopic resections. *Histopathology* **67:** 914–917.
48. Brehmenr, A. 2006. Structure of enteric neurons. In *Advances in Anatomy, Embryology and Cell Biology*. S. Rallison, Ed.: 4–5. Berlin: Springer.
49. Faussone-Pellegrini, M.S. & C. Cortesini. 1986. Preliminary observations on a neuromuscular complex present in the transition zone of the human esophageal muscle coat. *Arch. Ital. Anat. Embriol.* **91:** 63–70.
50. Faussone-Pellegrini, M.S. & C. Cortesini. 1985. Ultrastructural features and localization of the interstitial cells of

Cajal in the smooth muscle coat of human esophagus. *J. Submicrosc. Cytol.* **17:** 187–197.
51. Torihashi, S., H. Horisawa, & Y. Watanabe. 1999. c-Kit immunoreactive interstitial cells in the human gastrointestinal tract. *J. Auton. Nerv. Syst.* **75:** 38–50.
52. Faussone-Pellegrini, M.S. & L. Thuneberg. 1999. Guide to the identification of interstitial cells of Cajal. *Microsc. Res. Techn.* **47:** 248–266.
53. Thuneberg, L. 1982. Interstitial cells of Cajal: intestinal pacemaker cells? *Adv. Anat. Embryol. Cell Biol.* **71:** 1–130.
54. Keith, A. & L.L.D. Aberd. 1915. A new theory of the causation of enterostasis. *Lancet* **186:** 371–375.
55. Ambache, N. 1947. The electrical activity of isolated mammalian intestine. *J. Physiol.* **106:** 139–153.
56. Liu, L.W., E.E. Daniel & J.D. Huizinga. 1992. Excitability of canine colon circular muscle disconnected from the network of interstitial cells of Cajal. *Can. J. Physiol. Pharmacol.* **70:** 289–295.
57. Liu, L.W. & J.D. Huizinga. 1993. Electrical coupling of circular muscle to longitudinal muscle and interstitial cells of Cajal in canine colon. *J. Physiol.* **470:** 445–461.
58. Liu, L.W. & J.D. Huizinga. Canine colonic circular muscle generates action potentials without the pacemaker component. *Can. J. Physiol. Pharmacol.* **72:** 70–81.
59. Liu, L.W., L. Thuneberg & J.D. Huizinga. 1994. Selective lesioning of interstitial cells of Cajal by methylene blue and light leads to loss of slow waves. *Am. J. Physiol.* **266**(Pt. 1): G485–G496.
60. Huizinga, J.D., L. Thuneberg, M. Kluppel, *et al.* 1995. W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. *Nature* **373:** 347–349.
61. Knowles, C.H., R. De Giorgio, R.P. Kapur, *et al.* 2010. The London classification of gastrointestinal neuromuscular pathology: report on behalf of the Gastro 2009 International Working Group. *Gut* **59:** 882–887.
62. Tachimori, Y., Y. Nagai, N. Kanamori, *et al.* 2011. Pattern of lymph node metastases of esophageal squamous cell carcinoma based on the anatomical lymphatic drainage system. *Dis. Esophagus* **24:** 33–38.
63. Hirano, H., T. Nishigami, A. Okimura, *et al.* 2004. Autopsy case of congenital pulmonary lymphangiectasis. *Pathol. Int.* **54:** 532–526.
64. Jabbari, M., C.A. Goresky, J. Lough, *et al.* 1985. The inlet patch: heterotopic gastric mucosa in the upper esophagus. *Gastroenterology* **89:** 352–356.
65. Johns, B.A. 1952. Developmental changes in the oesophageal epithelium in man. *J. Anat.* **86:** 431–442.
66. Hamilton, J.W., R.G. Thune & J.F. Morrissey. 1986. Symptomatic ectopic gastric epithelium of the cervical esophagus. Demonstration of acid production with Congo red. *Dig. Dis. Sci.* **31:** 337–342.
67. Variend, S. & A.J. Howat. 1988. Upper oesophageal gastric heterotopia: a prospective necropsy study in children. *J. Clin. Pathol.* **141:** 742–745.
68. Peitz, U., M. Vieth, M. Evert, *et al.* 2017. The prevalence of gastric heterotopia of the proximal esophagus is underestimated, but preneoplasia is rare—correlation with Barrett's esophagus. *BMC Gastroenterol.* **17:** 87.
69. Maconi, G., F. Pace, L. Vago, *et al.* 2000. Prevalence and clinical features of heterotopic gastric mucosa in the upper oesophagus (inlet patch). *Eur. J. Gastroenterol. Hepatol.* **12:** 745–749.
70. Govani, S.M., V. Metko & J.H. Rubenstein. 2015. Prevalence and risk factors for heterotopic gastric mucosa of the upper esophagus among men undergoing routine screening colonoscopy. *Dis. Esophagus* **28:** 442–447.
71. Meining, A., M. Bajbouj, M. Preeg, *et al.* 2006. Argon plasma ablation of gastric inlet patches in the cervical esophagus may alleviate globus sensation: a pilot trial. *Endoscopy* **38:** 566–570.
72. Riddiough, G.E., S.T. Hornby, K. Asadi & A. Aly. 2017. Gastric adenocarinoma of the upper oesophagus: a literature review and case report. *Int. J. Surg. Case Rep.* **30:** 205–214.
73. Sarosiek, J. 2016. Does the healing of the esophageal mucosa improve the function of the esophageal submucosal and salivary glands? *Ann. N.Y. Acad. Sci.* **1380:** 155–161.
74. Sarosiek, J. & R.W. McCallum. 1995. What is the secretory potential of submucosal mucous glands within the human gullet in health and disease? *Digestion.* **56**(Suppl. 1): 15–23.
75. Yagi, K., A. Nakamura, A. Sekine & H. Umezu. 2006. The prevalence of esophageal cardiac glands: relationship with erosive esophagitis and nonerosive reflux disease (NERD) in Japanese patients. *Endoscopy* **38:** 652–653.
76. Hanada, K., K. Adachi, T. Mishiro, *et al.* 2015. Relationship between esophageal cardiac glands and gastroesophageal reflux disease. *Intern. Med.* **54:** 91–96.
77. Nie, L., H.Y. Wu, Y.H. Shen, *et al.* 2016. Esophageal submucosal gland duct adenoma: a clinicopathological and immunohistochemical study with a review of the literature. *Dis. Esophagus* **29:** 1048–1053.
78. Kiyozaki, H., T. Obitsu, D. Ishioka, *et al.* 2015. A rare case of primary mucoepidermoid carcinoma of the esophagus. *Clin. J. Gastroenterol.* **8:** 26–28.
79. Huang, Q. 2011. Definition of the esophagogastric junction: a critical mini review. *Arch. Pathol. Lab. Med.* **135:** 384–389.
80. Odze, R.D. 2005. Unraveling the mystery of the gastroesophageal junction: a pathologist's perspective. *Am. J. Gastroenterol.* **100:** 1853–1867.
81. Yassi, R., L.K. Cheng, S. Al-Ali, *et al.* 2010. Three-dimensional high-resolution reconstruction of the human gastro-oesophageal junction. *Clin. Anat.* **23:** 287–296.
82. Sharma, P., J. Dent, D. Armstrong, *et al.* 2006. The development and validation of an endoscopic grading system for Barrett's esophagus: the Prague C & M Criteria. *Gastroenterology* **131:** 1392–1399.
83. Alvarez Herrero, L., W. Curvers, F. van Vilsteren, *et al.* 2013. Validation of the Prague C & M classification of Barrett's esophagus in clinical practice. *Endoscopy* **45:** 876–882.
84. Compton, C.C. & American Joint Committee on Cancer. 2012. *AJCC Staging Atlas.* New York: Springer.
85. Srivastava, A., H. Appelman, J.D. Goldsmith, *et al.* 2017. The use of ancillary stains in the diagnosis of Barrett esophagus and Barrett esophagus–associated dysplasia: recommendations from the Rodger C. Haggitt Gastrointestinal Pathology Society. *Am. J. Surg. Pathol.* **41:** e8–e21.

86. American Joint Committee on Cancer. 2016. *AJCC Cancer Staging Manual*. New York, NY: Springer Science+Business Media.
87. Siewert, J.R. & H.J. Stein. 1998. Classification of adenocarcinoma of the oesophagogastric junction. *Br. J. Surg.* **85:** 1457–1459.
88. Fox, M.P. & V. van Berkel. 2012. Management of gastroesophageal junction tumors. *Surg. Clin. North Am.* **92:** 1199–1212.
89. Stein, H.J., D. Liebermann-Meffert, T.R. DeMeester, *et al.* 1995. Three-dimensional pressure image and muscular structure of the human lower esophageal sphincter. *Surgery* **117:** 692–698.
90. Miller, L., A. Vegesna, M. Ruggieri, *et al.* 2016. Normal and abnormal physiology, pharmacology, and anatomy of the gastroesophageal junction high-pressure zone: normal and abnormal GEJ high-pressure zone. *Ann. N.Y. Acad. Sci.* **1380:** 48–57.