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

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Abstract

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 that 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

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 on the outside, blood and lymphatic vessels and nerves, yet it has a unique job and it also has 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.

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

The esophagus is a 23–25-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 mucosa is lined by stratified nonkeratinized squamous mucosa. The lamina propria is composed of loose connective tissue that contains mucous glands in the distal portion. The esophageal muscularis mucosae is composed of longitudinally organized smooth muscle. The submucosa 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 muscularis propria 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 4th 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 tracheo-esophageal cleft. Similarly, Downregulation of Sox2 in the early foregut leads to EA/TEF [4]. The function of *Sox2* and *Nkx2.1 is regulated by s*everal 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 β-catenin. 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 pseudo-stratified columnar epithelium, which then becomes to ciliated near the mid-esophagus at 8 weeks of gestation. Starting from the 4th month of gestation, the ciliated epithelium gradually transits to squamous epithelium bi-directionally 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 muscularis propria, 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. Mutants lacking Foxp2 in a Foxp1 heterozygous background completely lose the striated muscle. Deletion of the Wnt signaling receptor Fz4 also affects the formation of the 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 the 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, 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 that down-regulation of squamous transcription factors (e.g., p63 and Sox2), up-regulation 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 Barrett's esophagus.[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 complexed 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: why do we have a squamous lining in our esophagus?

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 needs to be such that it can

withstand a reasonable degree of mechanical and/or chemical trauma. A simple stratified non-keratinizing 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 4-5 cell layer thick. It is impervious to any luminal contents. Stratum spinosum beneath the corneum, on the contrary, has very prominent desmosomes, and allows active transportation of molecules across the cell junctions. The stratum basalis, also known as the basal layer, is 2-3 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.

Is there a common set of reactions to injury to the squamous epithelium that occur as a result of several different stimuli? If so, what do these stimuli have in common?

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 (HIV/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 illicit 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, 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, and in response to several stimuli such as erosive GERD, non-erosive 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 and 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 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 Barrett's esophagus (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. A detailed discussion of the pathogenesis is beyond the scope of this review. Regardless of whether the stimuli are acid, bile salts or pancreatic enzymes that lead to recruitment of neutrophils,[22,23] or allergens that illicit an eosinophil-rich inflammatory response in genetically

susceptible individuals,[24] or the stimulus arises 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, dilatation of intercellular spaces 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, dilatation of intercellular spaces, 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 lamina propria and muscularis mucosae

The normal lamina propria (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 with Barrett's esophagus (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 muscularis mucosae (MM), which in patients with BE, undergoes duplication, fragmentation and expansion. This review will discuss the characteristics and prevalence rate of MM alterations, it's pathogenesis, histologic properties, and finally, the clinical implications of this phenomenon.

Muscularis mucosae 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 8 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 squamo-columnar junction, but distally at the level of the distal gastroesophageal junction, the superficial layer becomes attenuated and is replaced by fibrous tissue. A study by Abraham et al. showed similar findings.[29] In that study, 46 of 50 (92%) BE resections demonstrated "duplicated" MM, which involved between 5% to > 90% of the BE segment. However, in that study, 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, its 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" cancers. Hahn et al. evaluated the vascular and lymphatic properties of the mucosa and submucosa 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), intramucosal 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 submucosa of BE was not significantly different from the submucosa 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" (IMC), 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 regards 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 submucosa 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 classified depth of invasion into 4 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, intramucosal 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 1 (3%) patient with tumor that invaded into the LP/ inner MM, 0 patients with tumor that invaded the space between the superficial and deep LP, and 10 (33%) patients with tumor that invaded the true submucosa.[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 submucosa of the esophagus provides a flexible matrix, which serves as a cushion between mucosa and muscularis propria during peristalsis. It is also the regional routing center for blood and lymphatic flows. Histologically, the submucosa 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 submucosa.

A unique structure in the esophageal submucosa is the submucosal mucus gland. These are thought to be 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 Barrett's esophagus by confirming the esophageal origin of the sampled specialized columnar epithelium[35].

A rich lymphatic network is present in the lamina propria and is further concentrated in the submucosa. Several studies had suggested that lymphatics within the submucosa 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 muscularis propria/adventitia and regional lymph nodes. 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 submucosa, 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 submucosa into three equal layers) and the Paris Classification for stomach (submucosal invasion ≤ 500 μm as sm1, 500-1000 μm as sm2 and > 1000 μm 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 lymph node 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 muscularis propria, the submucosal response after mucosal injury plays an important role in stricture formation after endoscopic mucosal resection or submucosal dissection. In animal

models, starting from the 2nd day after a procedure, prominent inflammatory infiltrates are seen in the submucosa with a significant neutrophilic component. In the next two weeks, inflammation decreases and angiogenesis increases. By around 28 days after the procedure, in addition to dense fibrosis in the submucosa, the muscle layer also shows significant atrophy and fibrosis, which further reduces contractibility and flexibility of the esophageal wall.[47,48]

The muscularis propria

The esophageal muscularis propria, 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.

Are there specific diseases that target the muscularis propria?

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

Achalasia is associated with inflammation of the myenteric plexus of the m. propria. 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 m. propria 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 constitutes 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.

How can we distinguish between duplicated muscularis mucosae and muscularis propria in endoscopic mucosal resections?

The term "duplicated muscularis mucosae" (MM) refers to two layers of MM separated by connective tissue, a distinctive and common feature of Barrett's esophagus (BE). The more superficial (luminal) layer of MM delimits Barrett's mucosa. The deep MM is contiguous with the original MM of the squamous esophagus and continues caudally merging with the MM of the stomach (Fig. 1A). The lamina propria of the squamous esophagus is contiguous with the space between the duplicated MM. Below the deep MM is the submucosa. Invasion of adenocarcinoma into the duplicated MM space is interpreted as intramucosal carcinoma. Because of its patchiness, duplicated MM is seen only in a half to two thirds of the endoscopic mucosal resection (EMR) specimens.

It may be difficult to decide in the EMR sections whether adenocarcinoma invading beyond the only layer of MM is intramucosal or submucosal. When a second muscle layer is present at the deep margin, it may be challenging to differentiate the deep MM from the muscularis propria (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 that should be discussed promptly with the clinicians.

Recognition of the submucosa 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 submucosa are salivary-type glands, the adipose tissue and large-caliber muscular vessels. The vessels in the submucosa are larger, thicker, and

more tortuous and clustered than the vessels in the superficial lamina propria or the duplicated MM space. Using the presence of the salivary-type glands, the adipose tissue and large-caliber muscular vessels, Kaye et al. have recently demonstrated an excellent agreement in recognition of the submucosa in the EMR specimens, with kappa values in between 0.69 and 0.96.[49]

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.[50]

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 muscularis mucosae, 2) Henle's plexus – the deep component adjacent to the circular layer of muscularis propria, and 3) a less well defined intermediate plexus.[50]

The history of nomenclature of the enteric plexuses includes these details.[51] 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 muscularis mucosae). The plexus myentericus externus of Henle corresponds to the myenteric not to 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 submucosa, intermuscular and intramuscular layers of the esophageal wall.[50] ICCs are present in the mid esophagus associated with smooth and striated muscle and in the distal esophagus associated with smooth muscle. [52] ICCs are concentrated in the smooth muscle of the esophagus and within the lower esophageal sphincter. 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. [53,54] The ICC play an important role

in gut motility and serve as pace makers of motility. Frequent gap junctions between the ICC 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.[55] Evidence for the role of ICCs in gut motility and internal pace making activity has accumulated since their discovery.[56-58] The pacemaker activity is most concentrated in the ICCs in small intestine and stomach.[59,60] 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[61,62] or knocked out in a mouse model,[63] 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.[64]

The vascular and lymphatic supply of the esophagus: Why are there so many lymphatics in the lamina propria when no absorption occurs?

This lymphatic supply within the esophagus begins in the lamina propria and travels in the lamina propria and submucosa until large r 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 lymph nodes. The larger lymphatics penetrate the wall of the esophagus and each of these may drain up to about 40mm of esophageal submucosa.[65] 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.[66] 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. However, we do not actually know that; 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 lamina propria (lp) including the muscularis mucosae (mm), the submucosa (sm) between the mm and muscularis propria (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 any diseases that lead to vascular and lymphatic alterations? Does ischemic injury occur in the esophagus?

Congenital lymphangiectasia is incredibly rare[67] 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. These vascular or vascular-like diseases include the following:

- Acute esophageal necrosis (Gurvits syndrome, black esophagus, acute necrotizing esophagitis, esophageal infarction) Vascular/hypoperfusion: Shock, atheroma, vasoconstricting agents (cocaine), necrotizing arteritis
- Chemical injury: corrosives, acid, alcohol, medications
- Metabolic abnormalities: hyperglycemia, uremia, sepsis, lactic acidosis, anemia, hypoxia, hypoproteinosis
- Infections: CMV, Herpes, mycotic

- Mechanical injury, mostly iatrogenic: surgical manipulation, trauma from nasogastric tubes

- Co-morbidities: 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 and colleagues in their 1985 prospective endoscopy study, which encompasses most of its key clinicopathologic features.[68] 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. All lesions were located ≤ 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 cardia-type mucosa; no patient had intestinal metaplasia of the inlet patch. Inlet patches produced acid on pentagastrin stimulation. One patient (6.3%) had concurrent Barrett's esophagus. This brief summary will discuss the origin, prevalence, and clinical significance of the inlet patch.

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).[69] 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 to 21%)[70,71] 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 and colleagues 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%.[72] 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 6 times higher in the former.[73] 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 and colleagues reported a prevalence of 6.9% in volunteers.[74]

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 causes strictures, rings, webs, bleeding, ulceration, or perforation. In patients with attributable symptoms, inlet patches can be endoscopically ablated.[75] 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 Barrett's esophagus, 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 Barrett's. 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.[76]

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.[77] They secrete biologically active peptides, including trefoil factor family 3 (TTF3), epidermal growth

factor (EGF), transforming growth factor-alpha (TGF-alpha), and prostaglandin E2 (PGE2) to maintain the integrity of the squamous mucosa.[78] Submucosal glands also secrete a variety of defensive cell products; neutral and sialated 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 2–5 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 gastroesophageal reflux disease.[79] Hanada et al. performed endoscopic examinations on 2656 patients in search of cardiac-type glands on the proximal side of the gastroesophageal junction. 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 gastroesophageal reflux disease[80].

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[80]. These lobules consist of small aggregates (< 10) of glands invested in lamina propria 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 muscularis propria. 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 gastroesophageal reflux disease.[81]

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.[81] 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 Barrett esophagus 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.[82] 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 gastroesophageal junction and the lower esophageal sphincter

The gastroesophageal junction (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",[83] or where the esophagus and stomach meet (Z line). Histologically, it is defined as the junction of squamous and columnar mucosa.[83-85] 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.[83,86,87] 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".[88] 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 Barrett's esophagus,[83,88,89] staging of GEJ and stomach cancers,[88,90] and surgical classification and management of GEJ tumors.[91,92]

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.[93] This area corresponds to the lower esophageal sphincter (LES). The LES is not a true anatomic sphincter and this is a topic of continuous debate.[83,85,93,94] 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 anti-reflux barrier.[85,93,94] The phreno-esophageal ligament attaches the lower esophagus to the diaphragm and brings the distal esophagus back to

neutral position following peristalsis.[85] Proper function of these structures play an important role in swallowing and reflux/anti-reflux mechanisms.

To summarize, as can be seen from this detailed analysis, the esophageal wall, from mucosa through muscularis propria, 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, that is so short and so narrow has so many diseases intrinsic to it.

Competing interests

The authors declare no competing interests.

Author contributions

All authors contributed equally to the manuscript.

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