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Original Research

Non-goblet cell glandular structures underneath non-island squamous epithelium in columnar line esophagus on endoscopic tissues: Frequencies, forms, associations, and epithelial and mucinous characteristics

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ABSTRACT

Objective: Barrett's esophagus (BE) may possess variable cell lineages, leading to different phenotypes. Unusual glandular forms are currently being considered by some authors as important structures in the development of BE. The aim of this study is to analyze the frequencies, patterns, forms, and epithelial and mucinous characteristics of non-goblet cell glandular structures residing underneath the usual non-island squamous epithelium. **Materials and Methods:** Endoscopic biopsy tissues and reports of 299 cases with non-dysplastic and non-cancerous histology were examined and analyzed retrospectively. The cases were grouped according to their histopathological and endoscopic diagnosis and the presence of hiatus hernia. 6-8 serial tissue sections were obtained and stained with hematoxylin and eosin and alcian blue/periodic acid-Schiff (pH 2.5). The sections were examined under light microscope. **Results:** Abnormal glandular structures, which were deemed atypical for normal adult esophageal mucosa, were detected in the specimens. These abnormal patterns were grouped as esophageal glands proper and/or ducts-like ($n = 141$), island of ectopic gastric mucosal-like ($n = 73$), and dilated-hybrid glands-like ($n = 114$). Independent of hiatus hernia positivity, these structures were more frequent in columnar line esophagus cases than in chronic esophagitis group. Tall cell epithelium with light purple/purple-blue mucinous content was the most frequently observed type in these structures. **Conclusions:** Non-goblet cell glandular elements residing underneath the usual non-island esophageal squamous epithelium, whether isolated or forming a unit bearing immature properties, should be considered as metaplastic precursors and interpreted histopathologically as BE.

KEY WORDS: Barrett's esophagus, epithelium, glandular structures, mucin

INTRODUCTION

A columnar epithelial metaplasia observed between the distal esophagus and gastric cardia may not always represent columnar line esophagus (CLE). Further examination for a cardiac intestinal metaplasia must be performed for differential diagnosis [1]. The necessity is even more prominent in the presence of hiatus hernia. There is an ongoing debate on the normality of cardiac mucosa [2-4]. In one study, the histological development and changing mucinous characteristics of cardiac mucosa was thoroughly defined [5]. There are a number of differences between fetal and adult cardiac mucosa, which may help us build a scale to interpret the bizarre structures at distal esophagus.

Barrett's esophagus (BE) is usually associated with gastro-esophageal reflux (GER) and inflammation. The term "specified columnar epithelium" included in the definition of BE and/or CLE refers to a number of distinct phenotypes [6]. In addition, ductal-glandular growth theories have also helped to explain the histomorphology of BE [6-8]. Multifactorial interpretation of bizarre structures is a must. For this reason, a number of glandular structures have to be extensively examined and defined [7]. Cytochrome-c oxidase deficient glands [9], middle of glands [8], goblet cells [10-12], multi-layered epithelium [8,13,14], and esophageal ductal-columnar line changes [14,15] are known to be important markers for progression of disease and/or carcinoma.

Esophageal glandular structures in BE are traditionally classified into 3 types: Fundic, cardiac, and intestinal [14]. Currently, however, this classification is expanded as fundic, oxyntocardiac, non-goblet-cell columnar, Barrett's glands,

and Barrett's glands with paneth cells. In addition, formative metaplastic glandular structures on erosive replacement areas may change during the course of disease progression and depending on the Barrett's segment [7]. The presence of goblet cells within metaplastic structures should not be overlooked, as these cells are considered as a key indicator of progression toward BE. For this reason, the characteristics of goblet cell containing glandular-cryptic structures must be thoroughly analyzed and described. For example, Barrett's glands are clonal units [7], buried glands (metaplasia) are generally treatment-associated structures [14,16,17], and individual crypts are known to possess multiple uncommitted clones [18]. On the other hand, not goblet cells, but bidirectionality is the most important characteristic of the middle of glands [8]. Examination of non-goblet cell glands is considered important for the evaluation of disease progression [7]. The competition between glandular structures and/or stroma in BE is through non-canonical mechanisms [7,19]. Similar mutations that were observed in squamous epithelial island (SEI) and esophageal ducts, glandular acini and individual-isolated crypts [18], and dysplasia on crypt bases and surface epithelium [20] support this developmental model. Thus, colocalization of, and associations between the glands and metaplastic epithelial phenotypes comprise an interesting focus of study in BE (e.g., submucosal glandular hyperplasia and multi-layered epithelium-SEI [21-23]; superficial mucosal glands and specialized epithelium [24]; buried glands, residual metaplastic glands, and re-epithelialized squamous epithelium [16,25]). In general, SEIs and multi-layered epithelium are accepted to be highly associated phenotypes in BE [14,15]. To sum up, glandular structures in BE may have structural or spatial importance.

In this disease, ductal structures are analyzed through similar methods [26]. In normal tissues, esophageal ducts possess thin stratified cuboidal epithelium in lamina propria [27]. However, a vast variety of histopathological manifestations of these ducts (e.g., ductal epithelial hyperplasia in esophageal glands propers [EGPs]) have been defined in BE [27]. In addition, ciliated pseudostratified metaplasia of surface epithelium is interpreted as a precursor lesion [28]. Abnormal histomorphology on excretory ductal epithelium is interpreted as that it may be related to acid reflux [22], but some reports have suggested that this epithelial transition may also be caused by developmental disorders. According to some reports, for instance, ductal epithelium might be a precursor of multi-layered epithelium [29]. Furthermore, EGP and/or ducts were found to be increased in number in a BE associated adenocarcinoma case [30]. As a result, glandular and/or ductal structures in BE may be of great importance, in terms of their numeric, structural and spatial properties.

MATERIALS AND METHODS

Subjects

A total of 299 cases with a diagnostic consistency (i.e., histopathological diagnosis consistent with endoscopic diagnosis) were included in the study and analyzed retrospectively. Of the cases, 213 had BE/CLE and 86 had chronic esophagitis (ChrE) as confirmed by endoscopic examination and histopathological studies. Cases were grouped according to their diagnosis and presence of hiatus hernia [Figure 1A and B]. All cases were positive for GER symptoms. Tissue specimens were all free from dysplasia and/or carcinoma. All cases had at least one endoscopic biopsy tissue that contained usual non-island squamous epithelium (NISE). The frequencies of non-goblet cell glandular structures (NGCGSs) and epithelium characteristics were determined for all groups.

Histopathological Analysis

6-8 serial tissue sections with a thickness of 4 μm were stained with hematoxylin and eosin (H and E) and alcian blue/periodic acid-Schiff (AB/PAS) (pH: 2,5), and viewed under light microscope (Olympus BX46) for histopathological examination. Tissues were defined as squamocolumnar junction (SCJ) or covered by squamous epithelium according to their surface epithelium. Optimality of the tissues in terms of their adequacy for evaluation of the residing

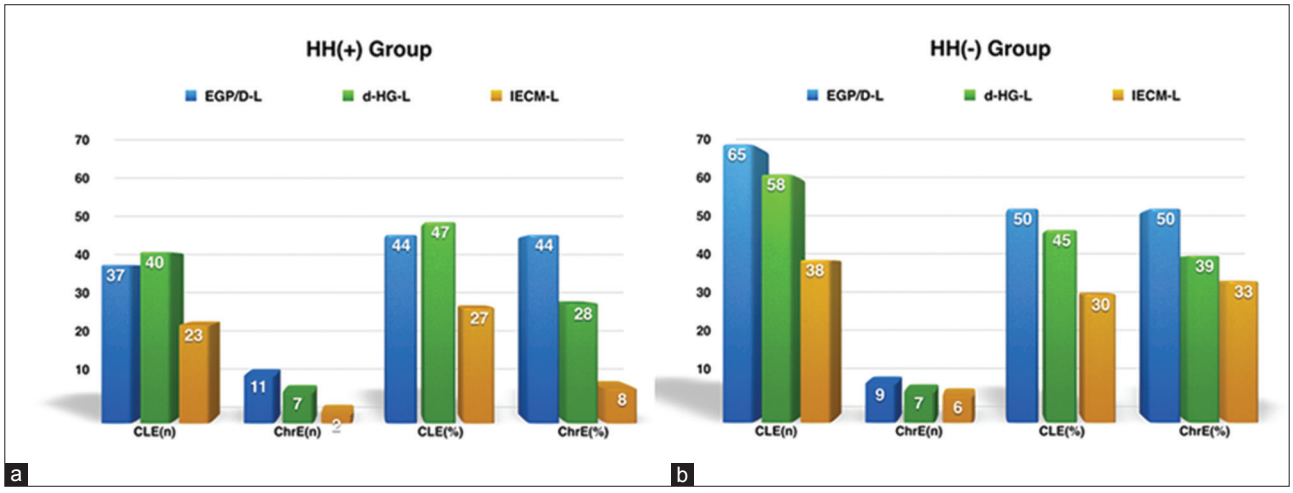

Figure 1: Distribution of non-goblet cell glandular structures underneath non-island squamous epithelium. (a) Hiatal hernia positive cases: 85 columnar line esophagus and 56 chronic esophagitis. (b) Hiatal hernia negative cases: 128 columnar line esophagus and 30 chronic esophagitis

glandular structures was assessed [1], and structures related to submucosal esophageal glands were excluded from the analysis. During histopathological examination, the presence of multi-layered epithelium [13,14], SEIs [9,14,26,31], crypt disarray and/or atrophy [14], esophageal ductal epithelial-columnar line changes [15], hybrid glands [14], buried glands [14,16,25], specialized intestinal epithelium [32-34], double muscular mucosa or muscular branching, and columnar or flat stratification of esophageal ductuli [15] were noted, and types of glands were determined [14]. The presence of at least 2 or 4 of the above (depending on the properties of the endoscopic tissue) was required for a diagnosis of CLE. Goblet cells positivity was not accepted as a criterion for CLE diagnosis [10-12,14,30,31,35-37]. Squamous epithelia were non-island, non-erosive, and non-regenerative. Afterward, NGCGSs underneath the NISE were determined. The forms, connection to superficial esophageal glands, and morphologic and mucinous epithelial characteristics of these glands were noted at serial-sections. The patterns of connections between NGCGSs and esophageal superficial glands were identified and classified as EGP and/or ducts-like [Figure 2A-C], dilated-hybrid glands-like (d-HG-L) [Figure 3A-C], and island of ectopic gastric mucosal-like (IECM-L) [Figure 4A-C]. Due to minor differences between the widely known definitions and the observed patterns, the term "-like" has been suffixed to their names, and the reasons are described below.

Types of Patterns and Forms of NGCGSs Underneath NISE

EGP/D-L

According to the classical definition of EGP and/or ducts, esophageal glands reside at submucosa [27]. However, the observed NGCGSs underneath the NISE of tubuloalveolar units were frequently connected with the superficial esophageal glands (the glands residing in lamina propria). Therefore, such NGCGSs were named EGP/D-L. Superficial glands were found to be of cardiac or oxyntocardiac types. Round shaped forms of these NGCGSs were named duct-like. Transition of duct-like form into cryptic form was observed in serial sections of some cases. In another group of cases, duct-like form was not connected to the esophageal glands even though the endoscopic tissues had a submucosa. Morphologically, these two groups of round-shaped glands were similar to the proximal glands of primitive stomach-cardiac mucosa and were interpreted as duct-like form in EGP/D-L pattern. Another structure showing EGP/D-L pattern was named glandular form, which was getting smaller or disappearing through consecutive serial sections, and they all had cardiac glandular epithelium [Figure 2C]. In fact, Takubo had defined these structures as EGPs [27].

d-HG-L

Distal glandular structures of hybrid glands are of cardiac type, according to the classical definition [14]. In our samples, d-HG-L structures showed two major deviations from this definition. First, those acinar structures were of oxyntocardiac type. And second, they showed a marked dilatation [Figure 3A-C]. The ostiums of those glands were rarely observable in our samples. However, the ones that we managed to observe were classified as d-HG-L form of EGP/D-L [Figure 2 Ba and Bb].

IECM

IECM is defined as a mucosal island having cardiac type acini and residing on the proximal esophagus. In addition, IECM epithelium may show ciliated pseudostratified metaplasia [27]. IECM like structures were often present in our tissue samples. These units formed a columnar epithelial area on the surface of squamous mucosa, some of which showed lateralization. Since some of these units had oxyntocardiac type glandular acini, they were different from the classical IECM definition. Thus, this pattern was named IECM-L [Figure 4A-C].

This study was approved by Kafkas University, Medical School Ethics Committee (approval number: 80576354-050-99/81, date: 15.10.2014; approval number: 17, date: 18.02.2016; approval number: 51, date: 08.04.2016).

RESULTS

The Optimality of the Tissue Samples in Terms of Allowing the Determination of the Connections between NGCGSS and Submucosal Glands

GER symptoms were present in 69% of all cases, and in 90% of the cases with CLE. Muscularis mucosa was observed in 62% of endoscopic biopsy tissues, allowing a proper examination of submucosal glands. NGCGSs with different forms and epithelium were primarily visible underneath NISE. In addition, these were frequently connected to the superficial esophageal

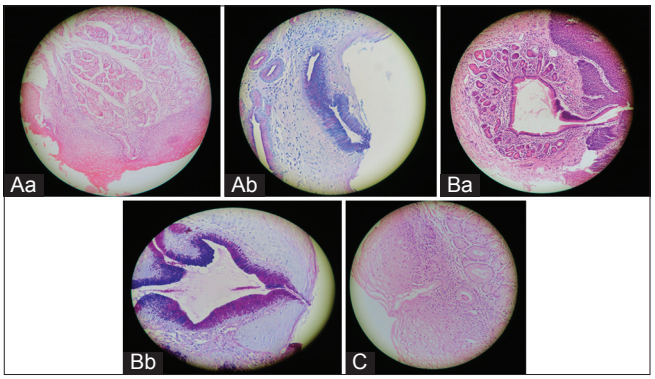

Figure 2: Esophageal glands proper and/or ducts like patterns. (A) - (Aa). In some cases, esophageal glands proper and/or ducts like pattern was connected to oxyntocardiac type esophageal glands. (Ab) Duct-like form is mostly lined by tall cell columnar epithelium containing light purple/purple-blue mucin (H and E, ×200; alcian blue/periodic acid-Schiff [AB/PAS] [2.5], ×400). (B) - (Ba) Dilated hybrid gland like form in esophageal glands proper and/or ducts-like (EGP/D-L) pattern. The ostium of dilated-hybrid glands-like form also is small. (Bb) Mucinous content of the epithelium is usually light purple/purple-blue (H and E, ×200; AB/PAS [2.5], ×400). (C) Glandular form in EGP/D-L that is isolated and lined by cardiac type epithelium (H and E, ×200)

glands and were building a tubuloalveolar unit, as observed through serial sections.

Pathological and Developmental Findings of NGCGSs

One may suggest that NGCGSs are elements of normal mucosa. However, some characteristics of these structures were similar to the distal esophageal and cardiac mucosa of fetal and neonatal periods. The frequency of NGCGSs was higher in CLE than ChrE, independent of hiatus hernia positivity [Figure 1A and B]. This result was important, because it was suggestive of the possibility that NGCGSs might belong to cardia.

EGP/D-L

Acinar glands residing in the tubuloalveolar units of EGP/D-Ls were usually of cardiac type, however some of them showed oxyntocardiac properties. The epithelium was dominantly non-ciliated tall cell columnar, which was similar to the proximal gland epithelium of the 17th gestational week [5] [Figure 2Aa] [Table 1]. The mucinous material contained in the tall cell columnar epithelium was mostly light purple/purple-blue when stained with AB/PAS [Figure 2Ab]. Focal pseudostratification or hyperplasia of the epithelium was rarely observed. Those areas were different from the defined ductal epithelial hyperplasia of EGP in BE [Figure 2Ab and Bb]. Finally, some of EGP/D-Ls were found to transform into cryptic form in 5-8% of the CLE cases, and resembled IECM-L as examination of the serial-sections revealed [Figure 5A].

d-HG-L

Another conspicuous pattern in CLE was dilated-hybrid gland-like (n = 104). The dilated glandular structures had metaplastic epithelium and were connected to cardiac or oxyntocardiac type superficial glands [Figure 3A and B]. Epithelium was generally tall cell columnar type in d-HG-Ls [Table 1]. Focal hyperplasia was present on tall cell columnar epithelium. Diffuse-focal light purple/aqua-light and/or deep-blue mucinous content within the epithelium was noteworthy. In addition, flat type epithelium was also observed in d-HG-Ls (n = 10, 18%), which was characterized by having a round-ovoid nucleus and a thin-compressed-eosinophilic cytoplasm [Figure 3C]. Mucinous content was usually stained diffuse or focal deep-blue with AB/PAS. However, diffuse pink staining of flat type epithelium was also noted in some cases.

IECM-L

Distal glands of IECM-L structures in lamina propria were of cardiac or oxyntocardiac type. They were similar to EGP/D-Ls at first glance but had a columnar epithelial area on squamous epithelium. In addition, their epithelium was different from that of EGP/D-Ls in that they were hyperplastic and of foveolar type on the surface, and of tall cell columnar type in lamina propria. In some cases, lamina propria contained low columnar or flat type epithelium. The interpretation of mucinous content in IECM-L was excluded since it showed a considerable variation from tissue to tissue and/or from section to section. However, it was obvious that the acidic mucinous content was not associated with the width of columnar epithelium on the surface [Figure 4B and C].

Multiple and coexisting patterns

In some cases, multiple structures of a single pattern or of more than one pattern together were detected (n = 58) [Figure 5A and B]. Multiplicity of one pattern was frequently encountered in d-HG-Ls, and this appeared as esophageal glandular hyperplasia (EGH). In addition, in CLE cases, coexistence of EGH and d-HG-Ls (59%) was also frequent.

EGH (n = 69, 32%) and/or separation (n = 62, 29%) was found in all CLE cases. The coexistence of EGP/D-Ls, d-HG-Ls, and ICEM-Ls presenting EGH and/or separation is summarized in Table 2 at CLE.

The most common epithelium was SCJ in samples, and distribution of NGCGSs of these tissues IECM-Ls (n = 33, 37), EGP/D-Ls (n:70, 57%), and d-HG-Ls (n:70, 57%).

DISCUSSION

There are various interpretation protocols and diagnostic criteria in BE [36-38]. The foremost aim is an early diagnosis of metaplasia, which may progress towards malignancy [39-41]. Unusual ductal-glandular structures associated with malignancy have been defined [15]. Our study focuses on these structures which reside on or associate with the usual squamous epithelium. NGCGSs underneath NISE, which were marked structures whether forming a unit or being isolated, were analyzed.

Diagnostic Usefulness of NGCGSs

Risk of being belongs to cardiac mucosa

Especially in IECM-Ls, analyzing NGCGSs alone could bring a risk of over-diagnosis of CLE, because these NGCGSs could be

Table 1: Maximal epithelium and mucinous properties of non-goblet cell glandular structures underneath the non-island squamous epithelium in Barrett's esophagus

Barrett's Eusophagus Cases	Maximal epithelium type (%)		Maximal mucin type in maximal epithelium type (%)	
	EGP/D-L (%)	d-HG-L (%)	EGP/D-L (%)	d-HG-L (%)
Hiatal Hernia (-) group	Tall cell columnar (88%)	Tall cell columnar (72%)	Diffuse-focal-light purple/purple blue (53%)	Diffuse-focal-light purple/aqua-light or deep blue (61%)
Hiatal hernia (+) group	Tall cell columnar (62%)	Tall cell columnar (60%)	Diffuse-focal-light purple/purple blue (61%)	Diffuse-focal-light purple/aqua-light or deep blue (55%)

CLE: Columnar line esophagus, HH: Hiatal hernia, EGP/D-L: Esophageal glands proper and/or ducts-like, d-HG-L: dilated hybrid gland-like

Table 2: Coexistence of esophageal glandular hyperplasia and/or esophageal glandular separation, and non-goblet glandular structures in cases of columnar line esophagus

NGCGSs	EGH *n*=69 (32%)	EGH+EGS *n*=19 (9%)	EGS *n*= 43 (20%)
EGP/D-L (n=117)	33 (48)	13 (68)	28 (65)
d-HG-L (n=99)	41 (59)	10 (53)	18 (42)
IECM-L (n=61)	18 (26)	5 (26)	11 (26)

EGP/D-L: Esophageal glands proper and/or ducts-like, d-HG-L: Dilated hybrid gland-like, IECM-L: Island of ectopic cardiac mucosa-like, EGH: Esophageal glandular hyperplasia, EGH+EGS: Esophageal glandular hyperplasia and separation, EGS: Esophageal glandular separation

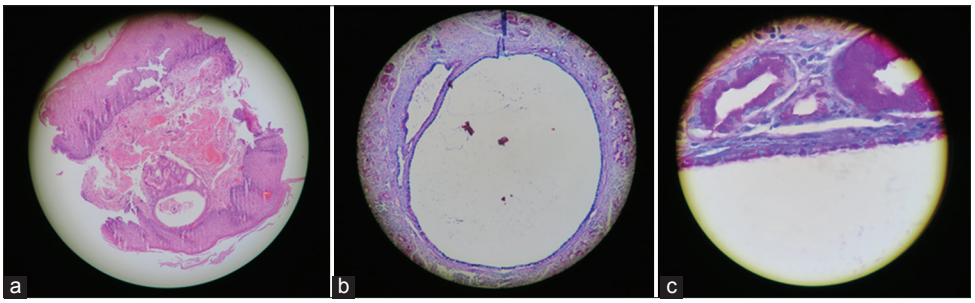

Figure 3: Dilated hybrid gland like patterns. (a) A solitary dilated-hybrid glands-like (d-HG-L) non-goblet cell glandular structure underneath the non-island usual squamous epithelium in chronic esophagitis (H and E, ×100). (b) The d-HG-L lined by low columnar epithelium. Frequently, mucinous content was of a color spectrum from light purple to light blue or aqua, and deep blue (alcian blue/periodic acid-Schiff [AB/PAB] [2.5], ×200). (c) Flat type epithelium in d-HGL that contained pink color mucin (AB/PAB [2.5], ×1000)

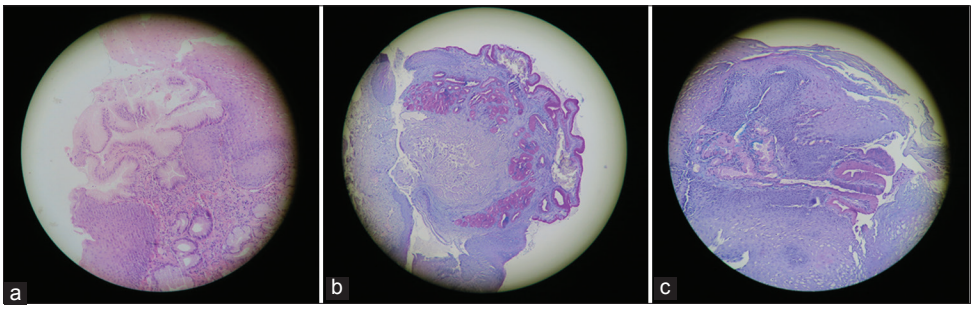

Figure 4: Island of ectopic cardiac mucosa like patterns. (a) Island of ectopic gastric mucosal-likes as units with columnar epithelium at the surface, that usually had cardiac type aciner glands (H and E, ×100). (b and c) Acidic mucin within the surface epithelium wasn't correlated with width of those (alcian blue/periodic acid-Schiff [2.5], ×100 and ×200)

originating from the gastric cardiac mucosa. This was avoided by looking for a lot of different phenotypes and requiring that at least 2-4 criteria were met. The possibility of cardiac derivation of these NGCGSs was also analyzed. IECM-L frequency was not increased by hiatal hernia positivity. Accordingly, the detected NGCGSs had a little likelihood of being of cardiac origin, and overdiagnosis risk in favor of CLE was minimal.

Unusual epithelial morphology in NGCGSs

Possible effects of low pH

The effect of low pH on epithelial morphology was another issue to be considered. Detectable surface openings of NGCGSs underneath NISE were also examined, to evaluate the possible effects of low pH on the epithelium. d-HG-L form had the smallest ostium diameter, followed by EPG/D-L ductal form, and the IECM-L and cryptic form EPG/D-L had the biggest. As a result, no obvious correlation between the ostium diameters and epithelium morphologies was noted.

Similarity between NGCGSs and immature epithelium

Epithelial morphology was similar in all pattern types. EGP/D-Ls were usually lined by single layer tall cell columnar epithelium at lamina propria, which was thought to be an indicator of immaturity. In addition, focal pseudostratified areas observed in glandular structures were interpreted as epithelial hyperplasia. This pseudostratification was first thought to be due to tangential section, because the possibility of tubular structures having a straight line was very low in endoscopic tissues. However, the similar focal pseudostratified epithelium was also observed in dilated areas on d-HG-Ls [Figure 2Bb]. Another comparison was made between tall cell columnar epithelium and ciliated pseudostratified metaplasia, which has been reported to be frequent at esophagogastric junction [28]. However, ciliary structures were not present on the surface, and the epithelium was predominantly single layered. Tall cell columnar epithelium at NGCGSs possessed some characteristics of cardiac mucosa of the 13th gestational week and proximal glands of the 1st neonatal week. For example, mucinous

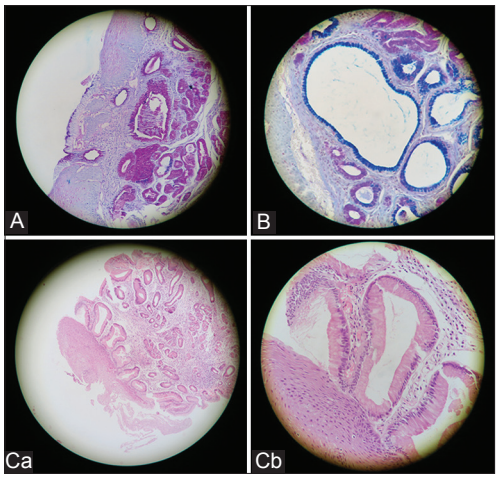

Figure 5: Multiplicity of non-goblet cell glandular structures underneath the non-island usual squamous epithelium. (A) Image of island of ectopic gastric mucosal-like with coexistence of esophageal glands proper and/or ducts-like patterns (alcian blue/periodic acid-Schiff [AB/PAS] [2.5], ×200). (B) Multiplicity of one pattern was usually observed in dilated-hybrid glands-likes of columnar line esophagus (AB/PAS [2.5], ×400). (C) - (Ca). A glandular trace underneath the squamous epithelial island. (Cb) Epithelium was very similar to tall cell columnar type (H and E, ×100 and ×400)

content of tall cell columnar epithelium which was stained light purple/purple-blue with AB/PAS was consistent with neutral-sialylated or neutral-sulphated mucinous content of the fetal and neonatal structures. In addition, although a metaplasia in response to an extrinsic effect should contain areas presenting the epithelium characteristics of former and metaplastic cells, a transitional zone from squamous toward the tall cell columnar type was absent in our samples. Probably, NGCGSs that possess EGP/D-L pattern comprise a novel conformation that originates from stem cells. Consequently, this particular group of EGP/D-Ls, which has a single layer tall cell columnar epithelium and a diffuse or focal light purple/purple-blue mucinous content, may be pre-lesions of metaplasia in CLE.

Flat epithelium was another structure of interest in that it was morphologically similar to the epithelium of early pit structure of primitive gastric mucosa of the prenatal and the early neonatal periods. Flat type epithelium was commonly present in d-HG-Ls and EGP/D-Ls. Usually, these cells were found to contain focal purple-blue mucinous material, but some of them had pink mucin with AB/PAS [Figure 3C]. Acidic mucin is a component of early pit structures of embryogenesis [5]. As a result, it was thought that this type epithelium in NGCGSs was related to intraluminal pressure within the glands.

The morphological differences in epithelium were thought to be mostly related to development rather than low pH.

Forms of IECM-L pattern as an indicator of disease progression

In IECM-Ls pattern, three points were important. First one was the formation of columnar epithelium at squamous epithelial surface. Essentially, this foundation is a replacement of squamous epithelium by columnar types. Cryptic forms, which were thought to be a transition from EGP/D-Ls to IECM-Ls (an intermediate form?) comprised the second important issue. Finally, multiple unique or combined patterns might be a marker of proliferation. Mutations in cell-cycle proteins were reported in BE [39,42]. Especially, side-by-side positioning of EGP/D-L and IECM-Ls has brought about the idea of transition between these two patterns [Figure 5A]. In some cases, the SEIs were formed by the juxtaposition of multiple IECM-Ls. Whereas in another group of cases, the columnar epithelium near these islands was extending underneath the SEIs, producing proliferated glandular traces and/or cryptic forms. This morphology was suggestive of a squamous and glandular epithelial competition. The glandular trace was lined by tall cell columnar and low columnar epithelium, and these were very similar to EGP/D-Ls [Figure 5Ca and Cb]. The mucinous properties of the regions showing IECM-L pattern were variable, rendering our discussions about intestinalization unfeasible. However, we could easily state that the distribution and density of acidic mucin within the surface columnar epithelium were not correlated with the length of the epithelium [Figure 4b and c]. The heterogeneity of the structures in BE is explained by multiple progenitor cells [42]. The IECM-Ls were one of the most heterogeneous NGCGSs underneath the squamous epithelium in this study.

d-HG-L pattern could be contained divergent cells

Another important pattern in CLE in terms of numeric and structural properties was d-HG-Ls, and they were usually lined by tall cell columnar epithelium. The mucinous structure was usually stained purple-deep blue or purple-light blue-deep blue with AB/PAS (pH:2.5). Sulphomucin is accepted as a specific indicator of incomplete intestinal metaplasia in BE [34]. In this study, mucinous content was examined only in terms of their neutral or acidic properties. However, the levels of mucinous transition toward acidic type were observed only in d-HG-Ls, even though they had a small ostium diameter. Nonetheless, d-HG-Ls showed characteristics of combined (divergent) metaplasia in CLE.

All types of NGCGSs underneath NISE were more prevalent in CLE than in ChrE. It has been thought that IECM-Ls were the progressed patterns of EGP/D-Ls, except for intestinalization. As an exception, d-HG-Ls did not show a transition of patterns. However, the highest degree of multiplicity of one pattern was observed in d-HG-Ls. In addition, step-by-step acidic transition of the mucinous material was only observed in d-HG-Ls. Among all types of NGCGSs underneath NISE, one of the most common epithelium types was non-ciliated tall cell columnar, which has a diffuse or focal light purple/purple-blue or deep blue stained mucinous content as stained with AB/PAS (pH:2.5).

As a final word, during histopathological studies, particularly of endoscopic biopsies that show only usual squamous

epithelium, detection of NGCGSs underneath the NISE could be interpreted as BE.

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Note:

We changed the paranthesis instead of bracket in reference citation [34] as per your replied query for AQ5. But we inserted the same reference citation 34 in the highlighted text in page no. 77, line no. 7 also for not changed the chronological order in the text. Kindly check if it is ok.