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Original Contribution

Ciliated columnar epithelium in the esophagus and gastroesophageal junction: A different perspective from study of a North American population

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ABSTRACT

An index case of ciliated columnar epithelium in a gastroesophageal (GE) junction biopsy identified in routine surgical pathology practice struck us as highly unusual. However, pathology literature, mainly from Asian populations, reports ciliated columnar epithelium in up to 40% of tissue samples from the upper GI tract. This was inconsistent with our pathology practice experience, so we initiated a local review of cases at our Canadian centre. 1048 consecutive tissue samples from the esophagus and GE junction were reviewed retrospectively and no ciliated epithelium was identified. This review included 1000× oil immersion microscopy of 22 cases with "multilayered epithelium". In 971 cases verified in prospective surgical pathology practice following identification of the index case, 3 additional cases of ciliated columnar epithelium were identified. The index case had ciliated pseudostratified columnar epithelium, resembling respiratory epithelium, and had strong, diffuse expression of TTF-1 by immunohistochemistry. In the other 3 cases, the cilia were located on the surface of a pseudostratified columnar epithelium, a multilayered epithelium, or a low columnar epithelium, all TTF-1 negative. Over a year later, the index case proved to have arisen from a bronchial-esophageal fistula. The other cases were not associated with a fistula. Our conclusion is that ciliated columnar epithelium is rare in Canadian adults (< 0.5% of patients). Ciliated epithelium due to a bronchial-esophageal fistula is exceptional, but something to consider if there is a suspicious clinical picture and TTF-1 expression. Other cases might represent a rare metaplastic phenomenon or remnant from fetal development.

1. Introduction

Although the human esophagus has a ciliated columnar epithelial lining at one stage of fetal development, ciliated epithelium is not typically described as part of the normal histology of the adult esophagus or gastroesophageal junction (GEJ) [1]. Thus, when we identified an area of ciliated columnar epithelium in a GEJ biopsy in routine surgical pathology practice (illustrated in Fig. 1) it seemed highly unusual, at least based on personal experience. However, there is a small body of literature that suggests that ciliated epithelium is relatively common in the adult stomach and esophagus [2-6]. In some of these studies, which are predominantly from Asian populations, ciliated columnar epithelium has been identified in up to 40% of upper gastrointestinal (GI) tract specimens reviewed [5].

We were skeptical of the high prevalence of ciliated columnar epithelium reported in the upper GI tract in the pathology literature. We undertook this study to independently determine the prevalence of ciliated columnar epithelium in the distal esophagus and GEJ in our North American (Canadian) patient population. Given a reported association between multilayered epithelium and cilia, we targeted cases with multilayered epithelium for detailed study with high magnification (1000×) oil-immersion light microscopy and immunohistochemistry for the respiratory epithelial marker TTF-1. We reviewed the clinical picture in cases with ciliated columnar epithelium.

2. Materials and methods

2.1. Index case

Ciliated columnar epithelium was identified in a biopsy of columnar mucosa from the GEJ at routine surgical pathology sign-out. Clinical

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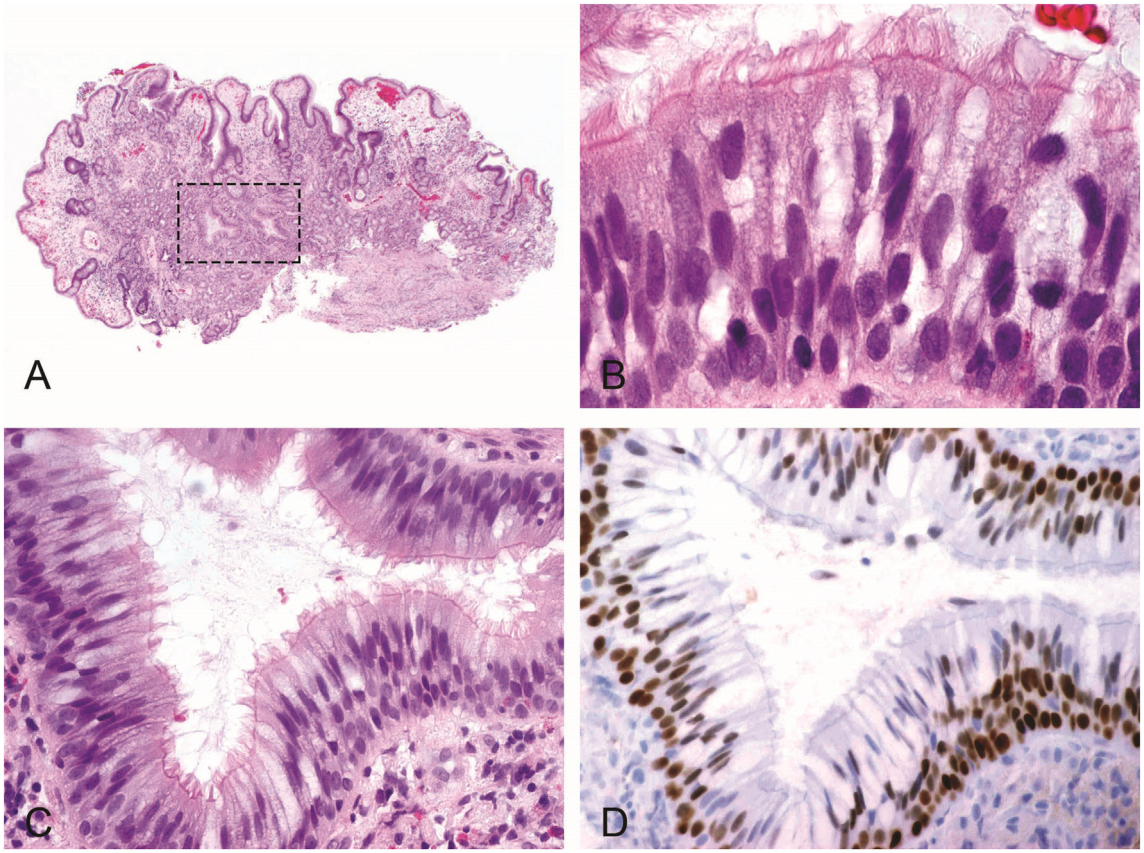

Fig. 1. In the index case, there is ciliated columnar epithelium in two gland or duct-like structures, highlighted with a box (Panel A, H&E, 20× magnification). Nuclear pseudostratification, terminal bars, and cilia are very prominent at 1000× magnification (Panel B) and easily visible at 400× magnification (Panel C). TTF-1 immunohistochemistry is strongly positive in the ciliated columnar cells (Panel D, 400× magnification), which later proved to have originated from a bronchial-esophageal fistula.

history and follow up biopsies from this patient were reviewed for a period of three years after the index biopsy.

2.2. Retrospective review of esophageal and gastroesophageal junction tissue for ciliated and multilayered epithelium

All consecutive tissue samples from the esophagus or GEJ from January 1, 2013 to March 31, 2014 were identified by electronic search of the anatomical pathology archives at the Queen Elizabeth II Health Sciences Centre (an academic adult tertiary-care centre in Halifax, Nova Scotia, Canada). The research ethics board of the Nova Scotia Health Authority approved the study protocol for the review. 1048 esophageal biopsies, endoscopic mucosal resections and surgical esophagectomies were identified and archived H&E slides were reviewed by light microscopy. In the screening review, slides were reviewed at 20–200× magnification, so cilia had to be clearly visible at 200× magnification to be identified in the study. All cases with multilayered epithelium were also identified, based on the defining criteria of an epithelium with multiple layers, having surface columnar cells and multiple underlying layers of flattened squamoid cells, as previously described [7].

2.3. Review of multilayered epithelium cases for cilia, using oil immersion microscopy

All 22 cases with multilayered epithelium identified in the initial slide review were analyzed for cilia at 1000× magnification using oil immersion microscopy. The multilayered epithelium, as well as esophageal ducts, foveolar epithelium, pyloric type glands, and oxyntic glands in these cases were carefully scrutinized at 1000× magnification for cilia.

2.4. Ciliated columnar epithelium in prospective routine surgical pathology practice

The last author of the study searched for ciliated columnar epithelium in prospective routine surgical pathology practice in a 3.5 year period, not overlapping the retrospective review. During this time period, this pathologist verified 971 surgical pathology cases sampling the GE junction or esophagus.

2.5. TTF-1 immunohistochemistry

The index case with ciliated columnar epithelium, three prospectively identified cases with ciliated columnar epithelium, 13 retrospectively identified cases with multilayered epithelium, nine cases sampling esophageal ducts, and two cases of ciliated fetal esophagus (archived tissue from fetal autopsy as a control) were selected for immunohistochemistry with TTF-1. The 20 cases with multilayered epithelium and/or esophageal ducts (2 cases had both ducts and multilayered epithelium) were also assessed for TTF-1 expression in the background foveolar epithelium, pyloric type glands of the cardia, and oxyntic glands, if present. Immunohistochemistry for TTF-1 was performed using the SPT24 clone (Leica Biosystems, Wetzlar, Germany) at a 1:100 dilution with a Ventana Benchmark automated system (Ventana Medical System Inc., Tucson AZ). The Cell Conditioner 1 solution (Ventana) was applied for 36 min for antigen retrieval. The ultraView DAB detection kit (Ventana) was applied. Human lung and thyroid tissue served as a same-slide positive control.

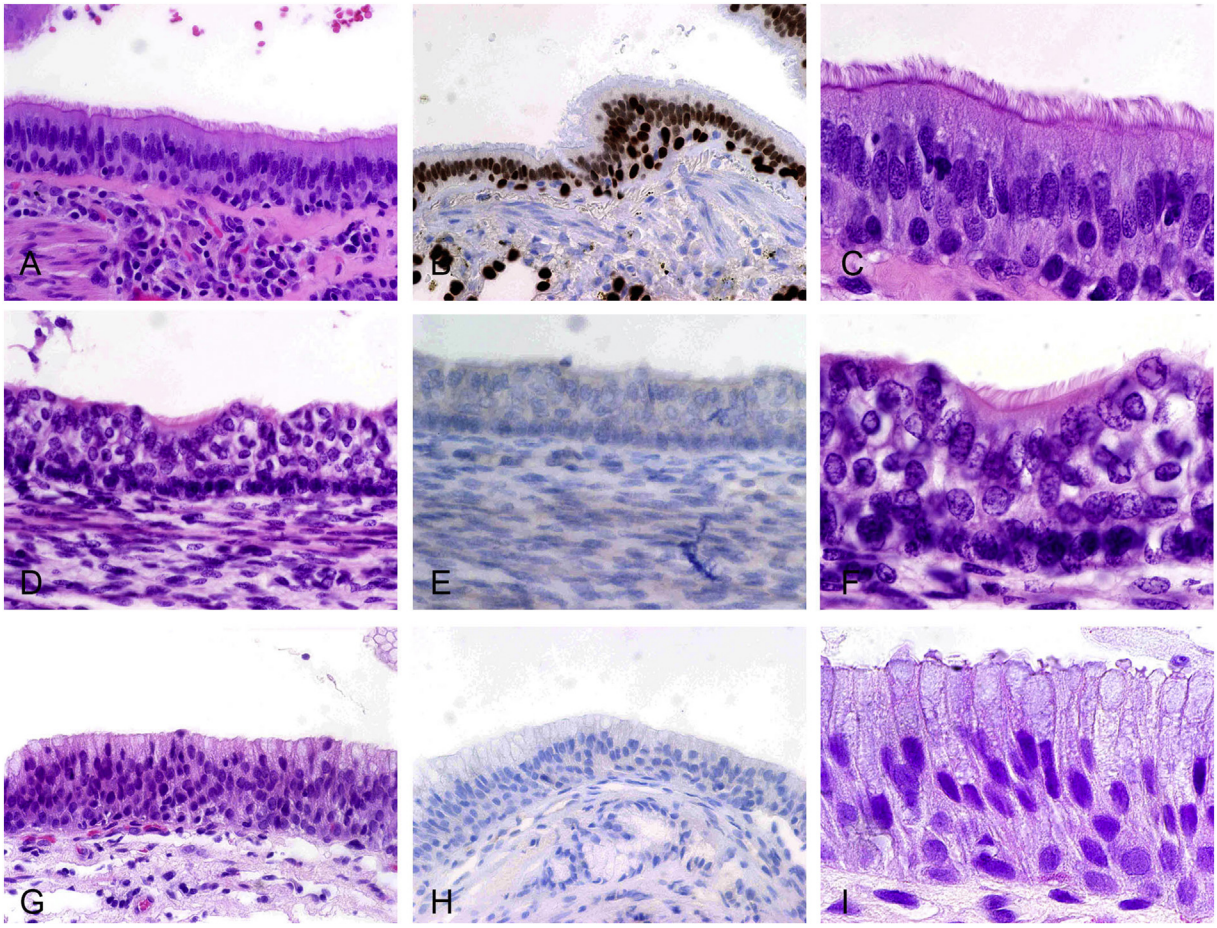

Fig. 2. A control case of respiratory epithelium from a lung bronchus illustrates the pseudostratified columnar epithelial cells of respiratory epithelium (Panel A, 400× magnification), their strong TTF-1 expression (Panel B, 400× magnification) and their prominent cilia (Panel C, 1000× magnification). The fetal esophagus has pseudostratified columnar epithelium with less voluminous apical cytoplasm than respiratory mucosa (Panel D, 400× magnification), no TTF-1 expression (Panel E, 400× magnification), and cilia that are easily visible but somewhat smaller than the adult respiratory mucosa (Panel F, 1000× magnification). A case of multilayered epithelium in the adult gastroesophageal junction also has multiple layers of nuclei (Panel G, 400× magnification), with no TTF-1 expression (Panel H, 400× magnification). Although we wondered if there were rare cilia on 400× magnification in this case, these seemed to be protrusions of cytoplasm at the apex of the cells when reviewed at 1000× magnification (Panel I).

3. Results

3.1. Index case

Pseudostratified columnar epithelium with prominent cilia was identified in GEJ biopsies from a 50 year old man in routine pathology practice (illustrated in Fig. 1). The pseudostratified epithelium included rare goblet cells, and had a striking resemblance to respiratory epithelium. The background non-ciliated epithelium did not have goblet cells. The ciliated epithelium involved two gland or duct-like structures in one slide (illustrated in Fig. 1) and reached the surface epithelium in another slide (not illustrated). Terminal bars and cilia were easily discernible at 100× magnification and all higher magnifications. The indication for endoscopy was investigation of iron deficiency anemia. The patient had a history of esophagectomy 10 years earlier for a gastrointestinal stromal tumor (GIST) of the proximal stomach. In the months following the initial biopsy, the patient developed hemoptysis, dysphagia, and a cough triggered by liquids, but not solids. Just over a year after the biopsy, a small fistula tract was identified between a bronchus and the GEJ by injecting an ulcer at the GEJ with saline, inducing a cough. The fistula tract was later excised surgically. An inflammatory fistula tract, partially lined by ciliated bronchial epithelium, was pathologically confirmed. There was no recurrent GIST. The fistula tract was presumed to represent a late complication of the prior esophagectomy.

3.2. Retrospective review of esophageal and gastroesophageal junction tissue for ciliated and multilayered epithelium

1048 consecutive tissue samples from the esophagus or GE junction were identified. 504 of these cases did not include a sample of benign columnar mucosa. These were almost always cases sampling squamous mucosa only, but some were biopsies containing only malignancy or only granulation tissue. The remaining 544 cases were reviewed for cilia in the columnar epithelium — none was identified. Multilayered epithelium was identified in 22 cases (4.0% of cases with columnar mucosa).

3.3. Review of multilayered epithelium cases for cilia, using oil immersion microscopy

High power (1000× oil immersion) light microscopy review of the 22 cases with multilayered epithelium revealed no cilia. Occasionally we had noted the presence of small surface protrusions on the apical surface of multilayered epithelium cells at lower magnifications, raising the question of rare cilia. However, the 1000× microscopy indicated that these were small cytoplasmic protrusions projecting above the surface. An example is illustrated in Fig. 2. Nine cases with esophageal

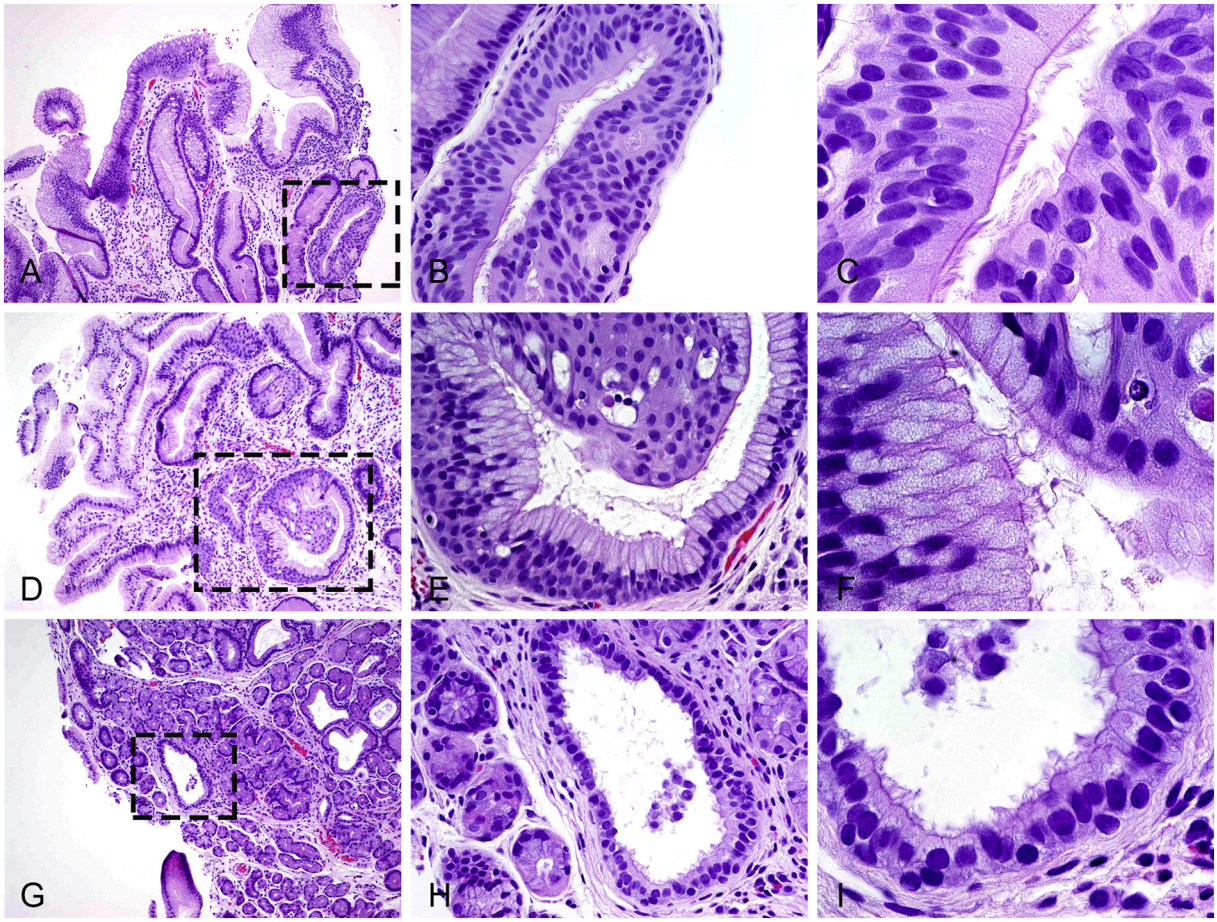

Fig. 3. Three additional cases of ciliated columnar epithelium in the GE junction or esophagus were identified in prospective routine surgical pathology practice over 3.5 years. The first case was identified in a duct-like structure highlighted by the box (Panel A, 100× magnification) and has pseudostratified columnar cells with terminal bars and cilia (Panel B, 400× magnification and Panel C, 1000× magnification). The second case is similar, and has some resemblance to multilayered epithelium, with surface columnar cells and basal squamoid cells (Panel D, 100× magnification, Panel E, 400× magnification, and Panel F, 1000× magnification). The third case is morphologically distinct, with the ciliated cells located in a dilated mucosal gland comprised of low columnar cells with short apical cilia (Panel G, 100× magnification, Panel H, 400× magnification, and Panel I, 1000× magnification). None of these cases express TTF-1 by immunohistochemistry (not illustrated).

ducts were also reviewed at 1000× magnification and none of the ducts had cilia. Foveolar, pyloric, and oxyntic type glands in 29 cases (with multilayered epithelium and/or ducts) were scrutinized at 1000× magnification. Again, cilia were undetectable.

3.4. Ciliated columnar epithelium in prospective routine surgical pathology practice

The last author of the study identified 3 cases of ciliated columnar epithelium in routine surgical pathology practice in a 3.5 year period after the index case was identified and following the time period of the retrospective cohort review. This pathologist verified 971 surgical pathology cases sampling the GE junction or esophagus in the 3.5 year period, indicating that cilia were present in 0.3% of cases. Two of these cases had cilia in tall columnar cells with nuclear pseudostratification, one of which resembled multilayered epithelium in one area (Fig. 3). There were no goblet cells in the ciliated columnar epithelium in these two cases, and there was no intestinal metaplasia in the background non-ciliated columnar mucosa. In each case, the ciliated cells were confined to a single duct-like structure. In the third case, cilia were present on the surface of short columnar cells without nuclear pseudostratification in a single gland (Fig. 3). There was intestinal metaplasia (Barrett's esophagus) in the background columnar mucosa, but no goblet cells in the ciliated mucosa. In the first two patients, the indication for endoscopy was dysphagia. In one patient the dysphagia was transient and resolved with proton pump inhibitor therapy and in the other it has persisted over several years without a clear cause or resolution. In the third patient the indication for biopsy was follow up for Barrett's esophagus. None of these patients were clinically suspected to have a fistula at any time in their history or 1–3 year follow up, and none had a history of thoracic surgery.

3.5. TTF-1 immunohistochemistry

The index case with ciliated epithelium had strong intensity nuclear staining for TTF-1 in > 90% of the ciliated columnar epithelial cells. The three cases with ciliated epithelium identified prospectively in routine surgical pathology practice were all completely negative for TTF-1. Thirteen of the 22 cases with multilayered epithelium were stained with TTF-1. There was weak to moderate intensity expression of TTF-1 in 5–20% of the cells in the multilayered epithelium in 3 of 13 cases (23%) tested. In the remaining 10 cases (77%), the multilayered epithelium was completely negative for TTF-1. None of the esophageal ducts stained positive for TTF-1 in 9 cases tested. Of 20 cases tested with foveolar type epithelium, the foveolar epithelium was completely negative, apart from one case with TTF-1 expression in < 1% of the foveolar epithelial cells. Pyloric glands had focal TTF-1 staining in 11 of 20 cases (55%), although the staining never exceeded 5% of the pyloric

Table 1
Previous studies identifying ciliated metaplasia in the upper GI tract.

Author, year	Number of cases	Specimen type reviewed	Anatomical location of ciliated epithelium	Proportion of cases with ciliated epithelium	Magnification/microscopy method
Raebum, 1951 [8]	Case report	Autopsy of esophagus	Distal esophagus	Case report	400×/light
Rubio and Kato, 1986 [2]	137	Gastrectomy	Antrum (pyloric glands)	35% (48/137)	1000×/light
Rubio and Serck-Hanssen, 1986 [11]	Case report	Gastrectomy	Antrum (pyloric glands)	Case report	1000×
Rubio and Antonioli, 1988 [12]	Case report	Gastrectomy	Antrum (pyloric glands)	Case report	1000×
Rubio, 1988 [10]	Case report	Gastrectomy	Antrum	Case report	1000×/light
Rubio et al., 1991 [5]	129	Gastrectomy	Antrum (pyloric glands)	40% (52/129)	1000×/light
Rubio et al., 1999 [3]	563	Gastrectomy	Cardia/corpus/antrum	34% (194/563)	400×/light
Rubio et al., 2005 [4]	3406 (1966 Atlantic, 1440 Pacific)	Gastrectomy	Cardia/corpus/antrum	15% total (5% − 98/1966 Atlantic; 29% − 418/1440 Pacific)	200×, 400× for detection; 1000× for examination/light
Takubo et al., 2005 [6]	16	Esophagectomy	Gastroesophageal junction	31% (5/16)	100×–400×/light 20,000×/electron
Schneider et al., 2011 [9]	Case report	Esophageal biopsy	Distal esophagus	Case report	400×/light

gland tissue present on the slide. Only a single case (1 of 8 tested) demonstrated TTF-1 staining in oxyntic glands, and this was found in < 1% of the oxyntic tissue in the sample.

4. Discussion

In our experience, ciliated columnar epithelium in the distal esophagus and GEJ of adults is rare. Based on the absence of ciliated epithelium in a retrospective review of 1048 cases and only 3 cases found in a prospective review of 971, the incidence of ciliated columnar epithelium in GEJ/esophageal biopsies is < 0.2% in our population. Even if only biopsies that include benign columnar mucosa are considered, the incidence remains < 0.5%. As outlined in Table 1, there is some controversy in the literature about the prevalence of ciliated columnar epithelium in the upper GI tract. In a series of publications, Rubio and Takubo and their colleagues have described ciliated metaplasia in up to 30–40% of cases examined [2,3,5,6]. In contrast, other authors have highlighted ciliated columnar mucosa in the upper GI tract as case reports, with emphasis on the uniqueness of this finding [8,9]. Our view is more consistent with the case report authors. There are a few possible explanations for the discrepancy. Studies by Rubio and colleagues [2,5,10] tended to emphasize high power (1000×) light microscopy for specimen review. This approach is very different from routine diagnostic surgical pathology practice, where magnifications above 400× are rarely used. Theoretically, 1000× oil immersion microscopy might detect tiny cilia that cannot be visualized at lower magnification. Although we only reviewed 22 cases at 1000× magnification, this did not reveal any ciliated columnar epithelium. We targeted the multilayered epithelium for the 1000× oil immersion review because the multiple layers of nuclei in multilayered epithelium resemble the nuclear pseudostratification in our index case and because Takubo et al. have identified cilia in 31% (5 out of 16) of cases with multilayered epithelium [6]. The 1000× oil immersion microscopy review highlighted some cytoplasmic protrusions at the apical surface of some cells in multilayered epithelium that might be a mimicker of cilia at lower magnification (see Fig. 2), but we were not convinced that any true cilia were present. Another possible explanation for the discrepancy between our study and others could be the anatomical location sampled and ethnic/geographic differences in study population. Although Takubo's study and ours focused on the GEJ, Rubio's studies focused mainly on stomach. Rubio and Takubo's studies had predominantly Japanese patient populations, in contrast to our Canadian population. Ciliated metaplasia in Tokyo and Matsuyama was reported to be present in approximately 30–40% of gastrectomies, while only 3–6% of cases were reported to have cilia in New York, London, and Stockholm [4,10-12]. This raises the possibility of a genetic or environmental influence.

The origin of ciliated columnar epithelium in the upper GI tract remains speculative and controversial. Our index case was clearly the unusual result of epithelial migration from a lung bronchus through a post-surgical fistula tract. Our other 3 cases of ciliated columnar epithelium had no clinical evidence of a fistula, suggesting some other mechanism. Some authors suspect that ciliated columnar epithelium in the upper GI tract is metaplastic. One theory put forward is that it might be an adaptive response to abnormal mucous retention, as a ciliated surface would facilitate mucous clearance [2-4,10]. Others have speculated that in the esophagus, a ciliated metaplasia might occur secondary to gastroesophageal reflux and might be part of the spectrum of multilayered epithelium and Barrett's esophagus [6]. Multilayered epithelium (also referred to as squamous-metaplasia like change by Takubo et al. [6]) is a hybrid type of epithelium comprised of basal squamoid cells with an overlying layer of columnar cells which has been associated with both Barrett's esophagus and gastroesophageal reflux disease [7,13-15]. In addition to finding frequent cilia, Takubo et al. noted patterns of cytokeratin expression (using CK4, 7, 8, 10, 13, 14 18, and 20) similar to bronchial epithelium in multilayered

epithelium [6]. We studied the bronchial epithelial marker TTF-1, but found minimal TTF-1 expression by immunohistochemistry in multilayered epithelium. Strong and diffuse TTF-1 expression should be the expectation in an epithelium with a true respiratory phenotype, but we only found that pattern in the index case arising from a bronchial-esophageal fistula. Our other 3 cases of ciliated epithelium were TTF-1 negative, which is similar to our finding in control cases of fetal esophagus. This pattern of TTF-1 immunonegativity speaks to a third possible origin for ciliated epithelium in the upper GI tract - an embryological or fetal remnant. By the 10th week of normal human embryological development once the esophageal lumen has formed, the primordial mucosa is comprised of ciliated columnar epithelial cells [1]. From the fourth month until birth, the ciliated epithelium is gradually replaced by stratified squamous epithelium that will then persist as the mature, adult epithelium [1]. Raeburn has described “islands of ciliated columnar epithelium” in the distal half of an adult esophagus at autopsy, and proposed this be a persistence of the fetal epithelium due to impaired developmental progression [8]. A similar hypothesis was put forward for pseudostratified ciliated columnar mucosa observed in the distal esophagus of an adolescent patient by Schneider et al. [9]. This patient was diagnosed in infancy with isolated congenital left pulmonary agenesis, and thus, the authors speculated that the ciliated columnar mucosa may also be congenital in origin rather than acquired.

In conclusion, our finding is that ciliated columnar epithelium in the esophagus and GEJ is rare (< 0.5% of patients) in Canadian adults in the assessment of surgical pathology using light microscopy at routine magnifications. If unequivocal ciliated epithelium is identified in the esophagus or GEJ, particularly within a pseudostratified columnar epithelium with strong TTF-1 expression, the rare possibility of a bronchial-esophageal fistula is something to consider and correlate with the clinical picture. However, rare cases of ciliated columnar epithelium in the GEJ and distal esophagus can occur without an associated fistula, and lacking TTF-1 expression. These cases have no apparent clinical significance and it is unclear if these cases represent a rare metaplastic phenomenon or a remnant from fetal development.

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Declaration of Competing Interest

None.

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