Mucin histochemistry of the columnar epithelium of the oesophagus: a retrospective study

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SUMMARY Reflux oesophagitis may be accompanied by replacement of squamous epithelium by columnar epithelium (Barrett's oesophagus). The columnar epithelium may resemble either gastric or intestinal mucosa, though ultrastructural and histochemical studies indicate that the intestinal type does not necessarily resemble normal small intestine. Twenty-two malignant and 10 benign oesophageal specimens were examined histologically and by mucin histochemistry in an attempt to classify the various types of columnar epithelium. An incompletely differentiated variant of intestinal metaplasia secreting sulphomucins was associated with well but not poorly differentiated adenocarcinomas ($p < 0.02$; Fisher's exact test). Similar findings have been described in the stomach and incomplete intestinal metaplasia may be related to disorders of growth and differentiation described in other sites such as the colon. These observations may help in the interpretation of oesophageal biopsies, possibly permitting identification of patients at risk of developing oesophageal adenocarcinoma.

It is generally accepted that adenocarcinoma of the oesophagus may arise from columnar epithelium of the distal oesophagus, acquired as a result of peptic reflux.¹ It is also accepted that the columnar epithelium may either be gastric or intestinal in type,¹ though histochemical and ultrastructural studies suggest that this classification may be an oversimplification.²⁻⁴ This paper poses the following questions: can mucin histochemistry help to characterise oesophageal columnar epithelium? Do well differentiated adenocarcinomas frequently arise in intestinal-type epithelium as in the stomach?⁵⁻⁶ Is an incompletely differentiated variant of intestinal metaplasia secreting sulphomucins associated with well differentiated adenocarcinomas, as in the stomach?⁷ Can mucin histochemistry help in the interpretation of oesophageal biopsies and the identification of a high-risk group of patients?

Material and methods

The material included 22 surgical resections for adenocarcinoma of the oesophagus, one resection for a benign peptic stricture showing columnar epithelium, and nine benign biopsies taken at oesophagoscopy purporting to include ectopic

columnar epithelium. This material was collected from patients attending Westminster Hospital since 1948. Thirteen of the tumours arose near the gastro-oesophageal junction, but were included in the study because the description and (if available) photograph indicated that the greater part of the lesion was contained within the oesophagus.

Serial sections were cut from each block and stained with haematoxylin and eosin, periodic acid-Schiff (PAS)-diastase (d), alcian blue (AB) pH 2.5 with PAS-d⁸ and high iron diamine (HID) with AB.⁹ The latter two combinations permitted distinction between acid and neutral and between sulfo- and sialomucins respectively.

Results

For the purposes of this study, tumours were divided into those with and without well differentiated areas. The latter resembled intestinal epithelium, since goblet cells secreting acid mucins and columnar cells with at least a partially developed brush border were represented. The columnar cells frequently differed from normal enterocytes however in showing mucin droplets in the apical cytoplasm (Fig. 1). Well-differentiated areas, including in particular the columnar cells, secreted mainly sulphomucins. Sialomucins and neutral mucins were the predominant

Fig. 1 Well-differentiated adenocarcinoma with columnar cells showing intermediate characteristics. At least a partially developed brush border is present yet the apical cytoplasm contains basophilic mucin droplets (identified as a sulphomucin on the serial sections). Haematoxylin and eosin $\times 100$ (original magnification)

Fig. 2a

Fig. 2b

secretion in poorly-differentiated tumours.

Benign columnar epithelium resembled either gastric or intestinal mucosa. Both fundic and cardiac glands (and intermediate forms) were observed in gastric epithelium and mucins were mainly neutral in type. Intestinal epithelium was characterised by barrel-shaped goblet cells secreting acid mucins. The intervening columnar cells rarely resembled normal absorptive enterocytes however. Their brush border

was variably developed and sometimes absent and the apical cytoplasm contained mucin droplets. When, on occasion, the columnar cells resembled normal enterocytes, the epithelium was termed complete intestinal metaplasia (IM) type I (though Paneth's cells were not observed). When the columnar cells secreted mucins the epithelium was designated incomplete IM type II. Type II IM was further subdivided into IIA when neutral mucins predomin-

Fig. 2c

Fig. 2 *An oesophageal biopsy from a patient with dysphagia (but no carcinoma) showing incomplete intestinal metaplasia, type IIA. (a) haematoxylin and eosin (b) periodic acid-Schiff-diastase (c) high iron diamine-alcian blue. Two populations of mucous cells, but no absorptive cells are present. The barrel-shaped goblet cells and the intervening columnar cells are PAS-positive. With HID-AB the majority of the goblet cells are AB positive only, indicating sialomucin secretion (grey) whereas the intervening cells are negative since they secrete neutral mucins. $\times 63$ (original magnification)*

ated and IIB when sulphomucins predominated (Figs. 2 and 3).

The incidence of all types of columnar epithelium bordering the well and poorly differentiated adenocarcinomas is given in the Table. Often two or more types of columnar epithelium occurred in the same case, explaining the discrepancy between the total number of observed types of epithelium (18) bordering the well differentiated tumours (15 cases). The distribution of columnar epithelium in the ten benign oesophageal specimens differed from the malignant cases, in that gastric and type IIA IM were the most common and extensive types of columnar epithelium. Five examples showed gastric epithelium, three type IIA IM and two mixtures of both. Type IIB IM occurred focally in three cases which also showed extensive type IIA change. Type

Fig. 3a

Fig. 3b

Fig. 3c
Fig. 3 Incomplete intestinal metaplasia, type IIB, taken from a specimen showing a well differentiated adenocarcinoma (not shown). (a) haematoxylin and eosin (b) periodic acid-Schiff-diastase (c) high iron diamine-alcian blue. Like IIA IM, two populations of mucous cells can be discerned. The goblet cells are strongly positive with PAS-D. Unlike type IIA IM, the intervening columnar (intermediate) cells show slight (but definite) PAS reactivity. However with HID-AB, the columnar cells are HID positive (black) indicating sulphomucin secretion. They also reveal at least a partially developed brush border. The majority of the goblet cells are AB positive with HID-AB, indicating sialomucin secretion (grey). Note the frankly dysplastic cells to the right. $\times 63$ (original magnification)

Incidence of various types of columnar epithelium in well and poorly differentiated adenocarcinomas

	Well differentiated adenocarcinomas (15)	Poorly differentiated adenocarcinomas (7)
Gastric epithelium	4	1
Intestinal metaplasia:		
Type I	2	0
Type IIA	3	0
Type IIB	9*	0

*Significant association of type IIB intestinal metaplasia with well differentiated adenocarcinoma ($p < 0.02$; Fisher's exact test).

I IM was observed as a focal change in only one specimen. Type I IM was therefore rare in both benign specimens and bordering tumours.

Discussion

The columnar epithelium of the oesophagus has been classified into two main types, either resembling gastric (fundic or cardiac) or intestinal epithelium.¹ Ultrastructural studies have shown that the intestinal type does not necessarily resemble normal small intestine.^{2,4} In place of normal absorptive enterocytes, there are columnar mucous cells bearing a partially developed microvillous border. Small intestinal enzymes may be demonstrated along the brush border, but in trace amounts.³ These cells appear to show features which are intermediate between a mucous and an absorptive cell and their counterparts have been observed in partial intestinal metaplasia (IM) in the stomach.¹⁰ They will be referred to as intermediate cells hereafter.

Mucin histochemistry showed the intermediate cells to differentiate along two principal lines, secreting either neutral or sulphomucins. Type IIB IM (with intermediate cells secreting sulphomucins) was associated with well but not poorly differentiated adenocarcinomas ($p < 0.02$; Fisher's exact test). It is also relevant that type IIB IM and well differentiated adenocarcinomas shared similar mucin profiles, with sulphomucins predominating. Furthermore, the columnar cells in well differentiated adenocarcinomas frequently resembled intermediate cells (Fig. 1). These factors suggest a histogenic link between type IIB IM and well differentiated adenocarcinomas. The negative correlation between type IIB IM and the poorly differentiated adenocarcinomas is evidence that the change is not merely secondary to tumour growth. The origin of the poorly differentiated adenocarcinomas is uncertain, since columnar epithelium (gastric type) was observed in only a single case. Congenital gastric heterotopias occur as small islands scattered throughout the oesophagus⁴ and could conceivably give rise to and subsequently be destroyed by a tumour. The submucous oesophageal glands are another possible source. Five of the poorly differentiated adenocarcinomas arose near the gastro-oesophageal junction and whilst the greater part of the tumour was contained within the oesophagus, it is also possible that a proportion may have been of gastric origin. The tumours were highly selected in all being amenable to surgical resection and this may explain the preponderance of well differentiated adenocarcinomas in the study.

It is interesting to compare the findings with recent observations made on the stomach in which type IIB IM (secreting sulphomucins) was also associated with well-differentiated or "intestinal" adenocarcinomas.⁷ Similar findings have been reported by others.¹¹⁻¹³ On the other hand, type IIA IM (secreting neutral mucins) was associated with benign gastric

lesions.⁷ Likewise in this study, type IIA IM was a frequent and extensive change in benign oesophageal biopsies, whereas type IIB IM occurred only focally. However the number of cases was too few to permit statistical analysis. Prospective studies are needed to show whether type IIB IM may serve as a useful premalignant marker.

The precise nature of the intermediate cell is not known for certain. It is tempting to compare it with its namesake in the generative zone of normal intestinal epithelium.^{14 15} The intermediate or oligomucous cell as described in detail in the colon, is an immature "undecided" cell showing both absorptive and secretory features.¹⁵ It is probably able to differentiate ultimately into either an absorptive or a mucous cell.¹⁴ Hyperplasia of intermediate cells occurs in a number of disorders of growth and differentiation in the colon including both adenomatous and metaplastic polyps¹⁵ and transitional epithelium bordering colonic carcinomas¹⁶ and a similar process could be operating in the incomplete intestinal metaplasias of the stomach and oesophagus. Clearly further detailed studies are needed to characterise this ubiquitous family of immature cells.

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