

Metaplasia in Barrett's esophagus: A specific phenotype?

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Barrett's esophagus (or columnar epithelium lined lower esophagus (CELLO) or "endobrachyoesophage" in French) is an acquired condition in which the squamous epithelium of the lower esophagus is replaced by a metaplastic glandular epithelium (1). Barrett's esophagus represents an abnormal healing of esophageal ulceration usually secondary to severe gastroesophageal reflux disease. Although the cell of origin of Barrett's esophagus is not firmly established, recent studies suggest that it is a multipotential stem cell of esophageal origin. The molecular mechanisms leading to metaplastic epithelium remain poorly understood. A number of gene products have been shown to be expressed during this process, including enzymes, growth factors and structural proteins. Of particular interest is the demonstration that CDX1, an intestine-specific transcription factor, may be important in the transition from normal esophageal epithelium to intestinal type metaplasia (2). It is also interesting to note that the expression of trefoil peptides (pS2 and hSP) may also participate in the delineation of the epithelial phenotype of Barrett's metaplasia (3).

Definition

The definition of Barrett's esophagus has been based mainly on the extent of glandular mucosa in the distal esophagus. Due to the difficulties of determining the length of Barrett's esophagus on endoscopy, a minimal length of 3 cm used to be the rule in most studies. However, recent reports have described short segment Barrett's esophagus (measuring less than 3 cm) and even "ultra-short" segment Barrett's esophagus, when the metaplastic epithelium was discovered on systematic biopsies of a Z line that appeared normal. Thus, there is a trend toward defining Barrett's esophagus based on purely histological criteria (1).

Histological features

Three types of epithelium can be present in Barrett's esophagus (4): fundic type, cardiac type, and specialized (or intestinal) type. Only the latter is distinctive and diagnostic for Barrett's esophagus. The specialized type of epithelium can be identified in long segment Barrett's esophagus in more than 90% of adult cases when a histological mapping with multiple biopsies is performed. In patients with high-grade dysplasia or carcinoma developed in Barrett's esophagus, the specialized epithelium is present in all cases on the surgical specimen (5). In children with Barrett's esophagus, specialized epithelium is less common, its prevalence increasing with age.

The repartition of the three types of epithelium in Barrett's esophagus is debated. Some studies have suggested that there is zonal repartition, from specialized to cardiac to fundic mucosa. In our experience, the three types of epithelium are usually intermingled in a mosaic pattern.

Cardiac and fundic mucosa in Barrett's esophagus usually have a disorganized architecture, a decreased number of glands, and show minor inflammatory changes.

The specialized mucosa has a villiform architecture, with characteristic epithelial cells present in surface, crypt and glandular epithelium. This epithelium is a mixture of at least four morphologically distinct cell types: goblet cells, columnar mucin-type "intermediate" cells, Paneth cells (present in most cases) and endocrine cells. Cells showing an unequivocal pattern of absorptive enterocyte (clearly defined brush border) are infrequent. A distinctive pattern of pancreatic metaplasia has also been described (6).

The goblet cells are usually large and distended by the accumulation of apical glycoprotein. On electron microscopy, the goblet cells have numerous apical mucin granules.

The columnar mucin-type cells have a straight lateral border and in ultrastructural studies, they have features both of gastric mucous cells (apical glycoprotein granules) and intestinal absorptive cells (apical microvilli). This intestinal phenotype is confirmed by the expression of brush border enzymes such as saccharase-isomaltase (7) or cytoskeletal proteins such as villin (8).

Mucin characterization

Histochemistry

Goblet cells produce acidic mucins, stained in blue with alcian blue or with the combined alcian blue-periodic acid-Schiff (PAS) stain. Using the combined high-iron diamine (HID)-PAS stain, the acidic mucin contained in goblet cells can be either sialomucin (blue) or sulfomucin (brown-black). The histochemical pattern of the columnar mucin-type cells is variable (9). They can produce neutral mucins, like gastric surface cells, or acidic mucins (sialo- or more often sulfomucins). It appears therefore that the histological and histochemical pattern of specialized Barrett's mucosa is analogous to that observed in incomplete intestinal metaplasia in the stomach (types II and III) (10).

Immunohistochemistry

The same type of results has been obtained with antibodies directed against carbohydrate chains and peptidic core of the mucins, with a predominant pattern similar to that of incomplete metaplasia of the stomach (11, 12). It has also been shown that in Barrett's intestinal metaplasia, goblet cells and columnar mucous cells both express a 40,000 Da antigen which are also expressed in the normal colonic epithelial cells but which are absent in small intestine epithelial cells (13).

Diagnostic problems

The presence of goblet cells, which are usually easily identified on an hematoxylin and eosin stain, is considered as mandatory for a diagnosis of Barrett's esophagus. It can be confirmed on an alcian blue stain. However, when a special staining is performed for acidic mucins, two diagnostic pitfalls have to be considered. Firstly, does the presence of alcian blue positive goblet cells always imply the presence of Barrett's mucosa, especially in patients with an irregular or even a normal-appearing Z line on endoscopy? The answer is no because, in a proportion of cases, these goblet cells are due to *Helicobacter pylori* atrophic carditis with metaplasia (14). Therefore, there is a need for new markers to distinguish between short segments of Barrett's mucosa and metaplastic carditis. A differential expression of cytokeratins 7 and 20 has recently been proposed (15). Secondly, is the presence of alcian blue positive acidic mucin in columnar cells a marker for Barrett's esophagus in the absence of goblet cells? The answer is probably no, as this pattern

has been observed in gastric cardia mucous cells in individuals with neither evidence of esophageal metaplastic epithelium nor symptoms or signs of gastroesophageal reflux disease (16).

In conclusion, Barrett's esophagus is defined histologically by its phenotype, similar to that observed in incomplete intestinal metaplasia of the stomach, with both goblet cells and intermediate metaplastic mucous cells. However, it may be difficult to distinguish between short segment Barrett's esophagus and carditis with intestinal metaplasia and there is a need for other markers in this situation.

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Intestinal metaplasia

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Gastric adenocarcinomas are subdivided into two main histological types with different epidemiological characteristics. The intestinal type of gastric cancer is preceded by a chain of phenotypical changes including *Helicobacter pylori* infection, chronic gastritis, atrophy, intestinal metaplasia and dysplasia. For the diffuse type of gastric cancer no similar precursors have been described.

H. pylori acquisition is the main cause of chronic gastritis in humans. The infection causes chronic active inflammation of the gastric mucosa in the majority of infected patients. In up to half of infected subjects, chronic gastritis progresses to atrophic gastritis and intestinal metaplasia. Various mechanisms are triggered that may contribute to the multistep pathogenesis of gastric cancer. Such mechanisms include inflammation-related cascades involving cytokines, free radical reactions, growth factors and their receptors. A novel member of the epithelial growth factor family, cripito, has been shown to be overexpressed in 35% of gastric cancers and is also seen in intestinal metaplasia (1). Numerous other factors may also play a significant role in this process, including overgrowth of bacteria in the hypochlorhydric or achlorhydric stomach, a high dietary intake of salt, nitrates or nitrites, a low intake of vitamins or micronutrients and an inherited genetic instability. The interplay of these factors may affect the cell genome and thus further influence progression towards gastric neoplasia. The molecular biology of gastric cancer has revealed a spectrum of gene errors that vary in type and extent between different histological types of cancer as well as between individual cases. The proposed pathway for the development at the genetic level of poorly versus well-differentiated gastric cancer reveals interesting differences (1). There is now evidence that the intestinal metaplasia or the gastric epithelium in atrophic gastritis reveals signs of the abnormal expression of various regulatory genes. Translocated promoter region-met rearrangements have been shown to occur in the earliest stage identifiable in the sequence, *i.e.*, in superficial gastritis, and K-ras mutations are associated with intestinal metaplasia (2). Thus, the mechanisms leading to mutation of the genes in epithelial cells may be triggered very early in the *H. pylori* gastritis sequence and atrophic gastritis and intestinal metaplasia may result from these processes.

It is likely that the nature of the disease complicating chronic *H. pylori* infection is determined by host and environmental factors while bacterial factors determine the magnitude of the risk of developing such disease. The pathogenesis of atrophy is still in part hypothetical. The rate at which atrophy develops has been addressed by investigators in different countries and has recently been summarized by Kuipers *et al.* (3). A range from 1.2-3.3% for the annual rate of development in populations is found as a whole. Conditions characterized by low rates of gastric acid secretion seem to be associated with a higher annual rate of development of atrophy. Furthermore, patients infected with cagA+ strains are almost four times more likely to develop antral intestinal metaplasia than cagA- patients (4).

Reversibility of a number of histological lesions can be achieved through *H. pylori* eradication. Reversibility has been shown in only the first step of the carcinogenesis sequence *i.e.*, in chronic superficial gastritis. Only limited, contradictory data are available on the potential reversibility of atrophy and intestinal metaplasia. Recent data would tend to suggest irreversibility. Forbes *et al.* (5) followed up 22 duodenal ulcer patients with failed *H. pylori* eradication and 32 duodenal ulcer patients with successful *H. pylori* eradication for a mean of 7.1 years. Glandular atrophy remained unchanged in both groups while the presence and severity of intestinal metaplasia was similar in both groups both initially and after follow-up. In a large prospective study, Van der Hulst *et al.* (6) investigated the course of atrophy and intestinal metaplasia after eradication in relation to cagA+ *H. pylori* strains. None of the 122 patients showed changes in the degree of atrophy and intestinal metaplasia after short-term follow-up or after follow-up for up to 18 months. Satoh *et al.* (7) con-