Barrett's Esophagus: Disregulation of Cell Cycling and Intercellular Adhesion in the Metaplasia-Dysplasia-Carcinoma Sequence

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Key Words

Barrett's metaplasia · Cell cycling · Cadherin · Catenin · Adhesion

Abstract

The incidence of both esophageal adenocarcinoma and Barrett's esophagus, a premalignant condition predisposing to this cancer, is rising rapidly. There is growing evidence that both of these conditions are related to the reflux of acid and bile into the esophagus. This results in inflammation and cell damage which initiates a sequence of events termed the metaplasia-dysplasia-carcinoma sequence in which the squamous epithelium is replaced by columnar epithelium exhibiting increasing degrees of dysplasia and overt malignancy. This sequence of events is underpinned by changes in cell cycling, such as accumulation of p16 and p53 mutations and increased cyclin D1 activity. Progression along this pathway is characterized by changes in intercellular adhesion, in particular, loss of adenomatous polyposis coli, reduced cadherin expression and increased catenin phosphorylation resulting in its nuclear translocation. Herein, we detail these molecular defects and propose how they may interrelate in an ordered progression in the development of esophageal adenocarcinoma.

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Introduction

Barrett's esophagus (BE) is best defined as a premalignant lesion in which the stratified squamous epithelium which normally lines the distal esophagus is replaced by metaplastic columnar epithelium [1]. Three types of columnar epithelium have been described: an intestinal type with villiform surface and mucous gland formation; an atrophic gastric fundus type, and a junctional type similar to that of the gastric cardia [2]. It is the intestinal type which carries the increased risk of malignancy with surveillance studies estimating the rate of developing adenocarcinoma at between 1 in 55 and 1 in 208 patient years, which is at least 40 times that of the general population estimate [3, 4]. Both the incidence of BE and that of esophageal adenocarcinoma are increasing rapidly [5, 6] to the extent that surveillance programs have been strongly advocated, although cost-benefit analysis of initial programs disputes their effectiveness [3, 7, 8]. Classical or long-segment BE in which at least 3 cm of the distal esophageal mucosa is metaplastic is reported in 10–15% of patients with gastroesophageal reflux disease (GERD) [9, 10], whereas short-segment BE is reported in nearly 20% of patients on routine endoscopy lists [7].

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0012-2823/00/0611-0001\$17.50/0

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Etiological Factors

The etiology of BE is not fully elucidated; however, it is clearly related to GERD in which gastric contents, particularly acid and pepsin, are regurgitated into the esophagus. Animal studies have demonstrated that acid denudation of epithelium can be followed by healing with columnar metaplasia [12] whilst human studies indicate that over 90% of patients with long-segment BE have increased esophageal exposure to acid on pH monitoring, with significantly increased exposure to pH 1–2. Recently the role of duodenogastric esophageal reflux has been considered, with the potentially noxious agents of trypsin and bile acids being refluxed from the duodenum to the esophagus [13]. It is notable that the taurine-conjugated bile salts, taurodeoxycholate and taurocholate, cause mucosal damage at pH 2, whereas unconjugated bile salts and trypsin are more harmful at pH 5–8 [14, 15]. Recent esophageal aspiration studies have shown increased levels of taurine-conjugated bile salts and acid in patients with BE and that the acid and bile salt reflux are temporally linked [16], suggesting that duodenogastric esophageal reflux in addition to GERD may be responsible for the mucosal damage that results in metaplasia. Additional risk factors for the development of BE of less significance include white race, male gender, obesity and being of affluent Western societies, whereas smoking, alcohol consumption and *Helicobacter pylori* infection are considered to be of minimal significance [17, 18].

Metaplasia-Dysplasia-Carcinoma Sequence

Although the colorectal adenoma-carcinoma sequence, and its underlying sequence of molecular mutations [19], is now a well-accepted model of cancer progression, a similar mechanistic pathway is only now becoming established for the development of esophageal carcinoma [20]. This involves a progression of mucosal changes through inflammation, metaplasia, dysplasia (low to high grade), carcinoma in situ and finally to invasive adenocarcinoma which may be referred to as the metaplasia-dysplasia-carcinoma sequence. The remainder of this review will outline what is known of the molecular defects that underpin progression along this pathway.

The exact mechanism of progression from inflammation to the development of Barrett's metaplasia is unclear. It is believed that Barrett's epithelium arises from pluripotent stem cells, although their exact location and origin remains a subject of debate [20]. The most popular theory

is that these stem cells arise from the papillae of inflamed squamous mucosa (de novo metaplasia theory). A second theory suggests that the stem cells are located in the esophageal glands and may selectively colonize the esophagus in times of squamous mucosal damage (duct cell metaplasia theory). In a third theory, cells found at the gastroesophageal junction may colonize either the gastric cardia or the distal esophagus (transitional zone metaplasia theory). These stem cells, when damaged by noxious agents such as bile and acid and in the presence of inflammatory infiltrate, differentiate down an alternative pathway to give rise to the Barrett's phenotype [2]. Metaplasia is associated with numerous genetic changes including allelic loss of chromosomes 17p, 18q and 5q. However, it is notable that the frequency of such loss is less in Barrett's metaplasia (14, 32, and 15%, respectively) than in esophageal adenocarcinoma associated with BE (75, 69, and 46%, respectively), supporting a progressive sequence of events [21]. Metaplasia is also accompanied by increased proliferative and apoptotic indices [22] and is associated with altered expression of multiple growth factors, heat shock proteins, atypical sulfated carbohydrates and increased levels of inducible nitric oxide synthase and cyclooxygenase 2 [23–25].

The development of dysplasia is characterized by a dysregulation of cell cycling. This is in part due to increased cyclin D1 expression and hypermethylation or mutation of p16 with mobilization of cells from G0 to G1 with subsequent accumulation in the G2 phase. This dysregulation of cell cycling is thought to reflect increased proliferative potential of epithelial cells and occurs early in the neoplastic progression with G2 accumulation being associated with progression to high-grade dysplasia [26]. There is also an increasing frequency of p53 mutations during the progression of dysplasia. These are uncommon in metaplasia in the absence of dysplasia, but are seen in 65% of low-grade dysplasia and 75% of high-grade dysplasia. The cell cycle is particularly sensitive to the effects of growth factors, particularly during early G1. The effect of abnormalities of cell cycling noted above may therefore be augmented by abnormal growth factor activity. Barrett's metaplasia is associated with elevated expression of both TGF- α and EGF, potent stimulators of cell proliferation which, acting through autocrine and paracrine mechanisms, increase the expression of multiple oncogene products such as c-fos, c-jun, c-myc and cyclin D [27–29], thus potentiating the increased proliferative potential of BE. Apoptosis, which may ameliorate the effects of increased cell proliferation, is markedly increased in esophagitis and low-grade dysplasia but appears to be inhibited in

Fig. 1. The interplay between adhesion molecules and cell cycle abnormalities and their relationship with histological changes in the progression through the metaplasia-dysplasia-carcinoma sequence.

high-grade dysplasia and adenocarcinoma through a mechanism independent of bcl-2 expression [22]. A further feature of the progression through dysplasia is an elevation in telomerase activity, hence ensuring the immortal growth characteristics and selective advantage of the Barrett's dysplastic cells over the normal epithelial cells [30].

Recent evidence has implicated the role of the cadherin/catenin adhesion complex and the adenomatous polyposis coli (APC) gene product (chromosome 5q) in the metaplasia-dysplasia-carcinoma sequence. The intracel-

lular levels of catenin are tightly regulated within epithelial cells such that any free cytosolic catenin forms a complex with axin, GSK-3 β and APC resulting in the rapid degradation of catenin. Phosphorylation of catenins or loss of APC may result in increased free cytosolic catenin. This free cytosolic catenin, which subsequently aggregates with Tcf/LEF transcription factors in the nucleus, results in increased transcription of several target genes including c-myc, c-jun, cyclin D1 and fra-1 [31, 32]. In BE, our group has shown increased tyrosine phosphorylation of β - and γ -catenin in response to TGF- α . Primary esophageal

cell cultures were stimulated with TGF- α , prior to immunofluorescence, immunoprecipitation and Western blot studies using antiphosphotyrosine antibodies as probes. These phosphorylated catenins can no longer bind to the cadherins resulting in impaired cadherin-mediated adhesion and accumulation of catenin within the nucleus [Tselepis et al., unpublished]. This leads to an inevitable downregulation of adhesion and may also lead to increased transcription of oncogenes such as c-myc and cyclin D1 resulting in further progression along the development to invasive adenocarcinoma.

It has been well documented that there is a downregulation of E-cadherin in invasive adenocarcinoma [33, 34] with an association between loss of E-cadherin and the occurrence of poorly differentiated tumors [35]. Whether this downregulation is in response to the nuclear translocation of β - and γ -catenin is not known, although murine studies would suggest that this is likely [36]. Interestingly in esophageal adenocarcinoma there appears to be a concomitant upregulation of P-cadherin [33]. Whether this is functional and can participate in cell-cell adhesion or precisely why these cancerous cells should upregulate this cadherin is not known. It has also been reported that there is reduced expression of a desmosomal cadherin, desmoglein 1, in esophageal adenocarcinoma, this protein is a major constituent of the other key cell-cell adhesion mechanism, the desmosome [33, 37].

Loss of cell-cell adhesion is a prerequisite for cell detachment from the primary tumor and the onset of metastasis. For both local invasion and metastasis to occur there must not only be detachment of cells from the primary tumor site by downregulation of cell-cell adhesion, but also disruption of the surrounding matrix rendering it permeable to cell penetration. Hence, for primary tumor cells to spread it is probable that elements such as matrix metalloproteinases (MMPs) are required to degrade the extracellular matrix. It is therefore not surprising to find that in esophageal cancer the production of a number of MMPs is elevated. Furthermore, it is of interest that EGF, which itself is upregulated early in the progression of Barrett's metaplasia, is able to trigger the production of MMPs [38–40].

This review therefore outlines what is currently understood of the molecular and genetic events surrounding the progression of Barrett's epithelium through to invasive esophageal cancer which are drawn together in figure 1. There are several major gaps in our knowledge which need addressing in the near future, not least of which is the mechanism by which esophagitis can lead to metaplasia. However, despite this, it is now becoming clear that the development of esophageal cancer is a multistep phenomenon involving genetic events that result in key abnormalities of cell cycle regulation, growth factor activity and intercellular adhesion mechanisms.

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