Genetic versus environmental interactions in the oesophagitis-metaplasia-dysplasia-adenocarcinoma sequence (*MCS*) of Barrett's oesophagus

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Abstract

The prevalence of Barrett's oesophagus has risen over a short time interval implying environmental in addition to genetic aetiological factors. Bile salt effects from duodenogastro-reflux are assuming increasing importance with deoxycholic and taurodeoxycholic acid being particularly associated with Barrett's oesophagus. The cellular biology changes appear to follow a progression from initial inflammation and oesophagitis to metaplasia and dysplasia through to adenocarcinoma. Mechanisms of restitution include epidermal growth factor mediated increases in epithelial thickness. This results in basal stem cells becoming superficially placed and exposed further to luminal refluxed bile salts. Immature stem cells result which undergo mutation to a metaplastic glandular phenotype with intestinal metaplasia. P53 mutation increasingly occurs in progression to dysplasia and carcinoma and may confer a survival advantage of these cell clones by delaying apoptosis. Cell cycling gene mutations occur with accumulation of cells in G2 phase. Disruption of cellular checkpoint mechanisms in the mitotic process result in loss of heterozygosity and aneuploidy including loss of the Y chromosome. Identical mutations between adjacent areas of dysplasia and adenocarcinoma supports clonal expansion as the mechanism of carcinogenesis. APC tumour supressor gene mutations are conserved in synchronous carcinomas in Barrett's dysplasia and are associated with ß-catenin accumulation in the nucleus and cellular migration with invasion. Cumulative genetic errors result in abnormal clones with metastatic or angiogenic potential. When a clone with malignant potential occurs adenocarcinoma can result completing the progression from inflammation to metaplasia and dysplasia through to adenocarcinoma. (Acta gastroenterol. belg., **2000**, 63, **18-21**).

Key words: Barrett's oesophagus, metaplasia, oesophageal carcinoma, genetic mutations, bile reflux.

Introduction

The rising incidence of adenocarcinoma in the distal oesophagus and gastric cardia over the last two decades has been paralleled by increasing prevalence of Barrett's oesophagus(BE) (1). This change over the short time span strongly implies environmental in addition to genetic factors are important in the aetiology of this condition (2). If these factors can be identified and successfully managed, treatment of the pre-malignant condition may be possible which is desirable as the associated adenocarcinoma carries such a poor prognosis. Acid reflux is a primary event in gastro-oesophageal reflux disease (3) but there is increasing evidence that other factors, particularly bile salt reflux are causal in BE (4). This may explain the lack of impact proton pump inhibitor therapy has achieved in the treatment of established BE.

The adenoma-carcinoma sequence (ACS) (5) is now established in colonic carcinoma with translation into clinical practice by polyp surveillance and endoscopic removal of pre-malignant lesions. A corresponding predictable succession of events is likely in oesophageal adenocarcinoma (Fig. 1) following an oesophagitis-metaplasia-dysplasia-adenocarcinoma sequence (MCS). Progression of BE to carcinoma occurring as rapidly as 3 years in patients with Barrett's oesophagus undergoing fundoplication surgery (6). The key to improving management of BE is successful identification and treatment of pre-malignant stages in the sequence. Cancers detected in surveillance programmes carry an improved prognosis of 35-45% 5 year survival compared with 5-15% in sporadic cancers even allowing for lead time bias (7). Recent advances in the understanding of molecular events in progression of Barrett's metaplasia to dysplasia and carcinoma give potential to identify high risk patients allowing for selected surveillance to improve effectiveness of screening and overall prognosis.

Duodeno-gastric reflux and Barrett's oesophagus

Gastro-oesophageal reflux of acid in uncomplicated BE is no greater than in patients with reflux oesophagitis indicating that factors other than acid reflux alone are important in the pathogenesis of BE (8). High levels of duodeno-gastric reflux and alkaline oesophageal reflux (9) imply a role of pancreatic secretions or bile salts. Deoxycholic acid (4) and taurodeoxycholic acid (10) have recently been demonstrated as the significant bile salts associated with BE. The presence of bile salts in refluxate is associated with BE rather than oesophagitis alone as BE patients have bile salt gastrooesophageal reflux either alone or mixed with acid (10). Quantity and quality of reflux appears important with the prevalence of BE approximately six times higher in patients with reflux symptoms for more than 10 years compared to less than 1 year (11). The length of BE is also associated with the degree of reflux as patients with long segment BE (> 3cm) have significantly decreased lower oesophageal sphincter pressures compared to patients with short segment BE (< 3 cm) (12).

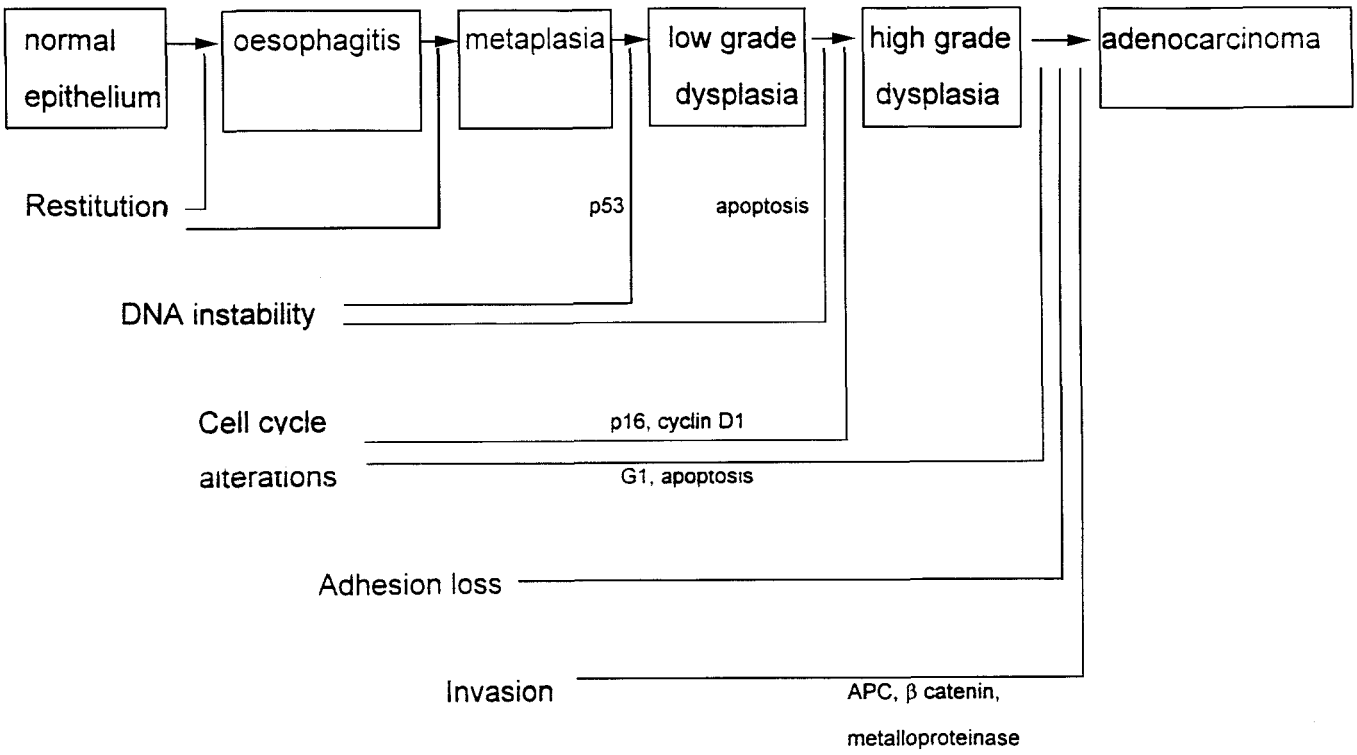

Fig. 1. — Barrett's metaplasia carcinoma sequance (MCS).
This is a schematic representation of molecular biological events during progression of MCS. These events may not necessarily occur in every case nor indeed in this exact order. However this sequence represents the best consensus of current hypothesis.

Oesophageal inflammation and Barrett's oesophagus

The relationship between oesophageal inflammation and Barrett's changes is complex with proximal inflammation but distal location of carcinoma. This is reflected in the cytokine response with increased pro-inflammatory IL-8 proximally and anti-inflammatory IL-10 distally (13). This confirms earlier studies showing another pro-inflammatory cytokine, TGF-ß in higher levels proximally (14). At a cellular level the initial epithelial damage from gastro-oesophageal reflux affects cells in the superficial compartment of the oesophageal mucosa resulting in increased cell loss. Regeneration with restitution of the epithelium occurs by increased cellular replication. As a protective mechanism the proliferative zone expands to maintain or increase epithelial thickness. The trophic effect of locally produced epidermal growth factor (EGF) (15) results in folding of the basal epithelium and papillae formation with the basal functional stem cells becoming more superficially placed and so more accessible to refluxed acid and bile toxins (16). When oesophageal mucosal restitution mechanisms are overwhelmed by excessive acid and bile injury the expression of epidermal growth factors results in increased immature stem cell production in place of native squamous epithelium.

Barrett's metaplasia

Immature stem cells from oesophageal squamous mucosa appear to undergo mutation with the development of a metaplastic glandular phenotype (17). This Barrett's metaplastic cell line is unique being pluripotential and giving rise to intestinal, gastric, Paneth and neuroendocrine cells (16). 3 individual types of metaplasia are recognisable, intestinal, junctional and cardiac although in vivo BE comprises of all 3 cell types in a mosiac pattern. The contribution of each cell type is affected by the type of refluxate (15) with predominant acid reflux leading to acid resistant cell lines as acid sensitive cells are damaged and undergo apoptosis. It has been postulated that bile reflux may promote bile resistant intestinal metaplasia and it is this mucosal type that predominates in acid suppressed patients. It is unclear at present which metaplastic cell line has the highest dysplastic and carcinogenic potential but suppressing acid alone will not prevent development of bile resistant cell lines.

The proliferation of Barrett's metaplasia is rapid with increased expression of multiple growth factors and inducible nitric oxide synthetase (18) and maximal colonisation of the oesophagus by metaplastic cells is rapid and can occur within 3 years of initiation (19).

Barrett's dysplasia

The progression of metaplasia to dysplasia in Barrett's oesophagus is associated with a number of cellular changes including chromosomal mutations, cell cycling alterations and cell adhesion abnormalities. The development of high grade from low grade dysplasia is likely to result from a number of these cellular events with carcinoma only resulting when a particular sequence of cytogenetic combinations occurs. The progression of low to high grade dysplasia and carcinoma is reflected in the finding that high grade dysplastic lesions have a 4 to 8 fold increased risk of developing carcinoma compared to low grade dysplasia (20). High grade dysplasia appears to represent an irreversible terminal event as 50% of high grade dysplastic lesions are immediately adjacent to adenocarcinoma (21). Identical mutations of the p53 tumour suppressor gene between these areas supports clonal expansion rather than multifocal field change as the mechanism of such carcinogenesis. p53 mutations occur increasingly during MCS and are found in 5-10% of cases with indeterminant dysplasia, 65% of cases with low grade dysplasia, 75% of cases with high grade dysplasia and up to 90% of cases with adenocarcinoma (22). p53 may effect the proliferation / apoptosis ratio allowing delay of apoptosis in dysplastic cells with conferred survival over native squamous epithelium (23).

Cell cycling is altered with reduced regulatory controls induced by mutations in the cell cycle genes. Mutations are found in p16 (chromosome 9p21), increased cyclin D1 expression and accumulation of cells in G2 phase (24). These cell cycle changes are associated with alterations in the expression of growth factors, including TGFα (25) and stimulation of the epidermal growth factor receptor.

Alteration in chromosomal number occurs by disruption of cellular check-points in the mitotic process resulting in loss of entire chromosomes with loss of heterozygosity and aneupoloidy (26). The Y chromosome in particular is lost in the Barrett's MCS and is absent in 9% of Barrett's metaplasia, 38% of indefinite dysplasia and 100% of high grade dysplasia (27). The significance of this is uncertain but the male preponderance of Barrett's oesophagus and oesophageal adenocarcinoma is interesting.

Adenocarcinoma in Barrett's oesophagus

Genetic mutation of the APC tumour suppressor gene with loss of heterozygosity occurs in dysplastic clones of Barrett's cell lines (28). This is associated with reduced ß-catenin degradation and subsequent accumulation. ß-catenin has been shown to accumulate with transition factors in the nucleus and can facilitate epithelio-mesenchymal transition with associated migration and invasion (29). Such genetic alterations are found to be conserved in synchronous invasive carcinomas in Barrett's dysplasia (30). Further mutations result in expression of metalloproteinases (31) and tumour cell motility factors resulting in failure of cells to adhere to neighbouring cells. Cumulative genetic errors result in clones of abnormal cells and increase the population of cells with metastatic or angiogenic potential. With the development of an unsuppressed malignant clone of cells adenocarcinoma results and the sequence of progression from oesophagitis, metaplasia and dysplasia through to adenocarcinoma is complete.

Conclusions

The metaplasia-carcinoma sequence in Barrett's oesophagus involves environmental factors particularly bile salt reflux via duodenogastric reflux which initiates cellular damage at the squamo-columnar junction. Marked inflammation results with mechanisms of restitution overwhelmed. Immature stem cells can result with intestinal metaplasia and the onset of Barrett's oesophagus which include bile resistant cell lines. Chromosomal mutations, cell cycling alterations and cell adhesion abnormalities are associated with progression of metaplasia to low grade and then high grade dysplasia. APC tumour suppressor gene mutations and ß catenein accumulation accompany the development of adenocarcinoma. The progression of inflammation with oesophagitis through metaplasia and dysplasia to adenocarcinoma then completes the metaplasia adenocarcinoma sequence (MCS).

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