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TOPIC HIGHLIGHT

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Risk factors for neoplastic progression in Barrett's esophagus

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Abstract

Barrett's esophagus (BE) confers a significant increased risk for development of esophageal adenocarcinoma (EAC), with the pathogenesis appearing to progress through a "metaplasia-dysplasia-carcinoma" (MDC) sequence. Many of the genetic insults driving this MDC sequence have recently been characterized, providing targets for candidate biomarkers with potential clinical utility to stratify risk in individual patients. Many clinical risk factors have been investigated, and associations with a variety of genetic, specific gastrointestinal and other modifiable factors have been proposed in the literature. This review summarizes the current understanding of the mechanisms involved in neoplastic progression of BE to EAC and critically appraises the relative roles and contributions of these putative risk factors from the published evidence currently available.

Key words: Barrett's esophagus; Esophageal adenocarcinoma; Metaplasia-dysplasia-carcinoma; Neoplastic progression; Risk factors

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INTRODUCTION

Barrett's esophagus (BE) describes a condition where native esophageal stratified squamous epithelium is replaced by metaplastic columnar epithelium, with cephalad displacement of the squamocolumnar junction. BE represents the only identified precursor lesion and most important risk factor for esophageal adenocarcinoma (EAC)[1]. Patients with BE have an estimated 30- to 125-fold greater risk of developing EAC than the general population[2]. A systematic review of 27 studies suggested annual progression rates of 0.5%[3], whereas a review of 8 UK studies by Jankowski *et al*[4] showed cancer risk of 1.0% per year.

BE PATHOGENESIS AND MECHANISMS OF NEOPLASTIC PROGRESSION

BE is an acquired condition where healing from esophageal mucosal injury [typically triggered by gastro-esophageal reflux disease (GERD)] is metaplastic, with replacement of damaged squamous cells by columnar epithelium. Ordinarily, esophageal healing involves regeneration of squamous cells; it remains unclear why the response is metaplastic in some individuals, since only a minority of patients with GERD develop BE. Progression of BE to EAC occurs by a metaplasia-dysplasia-carcinoma (MDC) sequence. Metaplastic columnar epithelial cells are predisposed to genetic damage with potential for developing

dysplasia[5]. Dysplasia represents a histological spectrum from low- to high-grade, defined by degree of cytological and architectural disruption present, with genetic instability resulting in progressive acquisition of genetic abnormalities towards a frankly neoplastic phenotype. These can be considered within the framework of Hanahan and Weinberg's[6] model of "cancer hallmarks" necessary for carcinogenesis, whereby cancer cells must acquire growth self-sufficiency, insensitivity to anti-growth signals, avoidance of apoptosis, limitless replicative potential, sustained angiogenesis, and invasive and metastatic potential[7].

Many genetic insults conferring these advantages in the BE MDC sequence have been characterized. Initiating events probably involve genes regulating cell cycle progression, notably *p16*. Mutations, loss of heterozygosity (LOH) or promoter hypermethylation (i.e. silencing) of *p16* have been identified in 80% of BE, whilst *p16* hypermethylation correlated with the degree of dysplasia in some studies[8]. Additional changes identified include upregulation of cyclins D1 and E, transforming growth factor-α and epidermal growth factor (EGF), each contributing towards growth autonomy[9,10]. These mutations should trigger apoptosis *via p53*-dependent pathways. However, subsequent accrual of *p53* lesions confers resistance to apoptosis, and has been identified in 52%-93% of EACs (compared with 1%-5% non-malignant BE cell lines)[11]. Inactivation of *p53* increases clonal genomic instability, predisposing to widespread DNA changes and evolution of ploidy lesions, late events in cancer progression. Many other genetic and molecular alterations have been described[8,9,12-64] (Table 1).

The concept of a linear, stepwise evolution of tumor suppressor gene mutations in which clonal expansion of a solitary mutated clone expands to fill the entire Barrett's segment has been termed the "selective sweep to fixation" model. However, an alternative model has been proposed by Leedham *et al*[65], who performed genetic analysis of individual crypts rather than a flow purified whole biopsy specimen. This technique permitted identification of certain mutations masked by whole biopsy segment analysis (attributed to dilution effect of the normal stroma on whole biopsy analysis), whilst also revealing a greater degree of genotypical and phenotypical heterogeneity within the same biopsy sample than previously appreciated. The demonstrated lack of a single founder mutation present in every crypt suggested that the clonal expansion arose from multiple independent clones rather than a single common founder mutation[65,66] (Figure 1).

This enhanced understanding prompted research into > 200 candidate novel biomarkers of disease progression in BE/EAC. Several, including 17p LOH, cyclin D1, tetraploidy and aneuploidy, have undergone phase 3/4 validation and in future might have clinical/prognostic utility as intermediate markers of progression[67]. However, Leedham's recent findings call into question the reliability of "surveillance" biomarker identification *via* genetic analysis of whole biopsy specimens, since minority clones within the sample (harboring neoplastic potential) might not be detected[65].

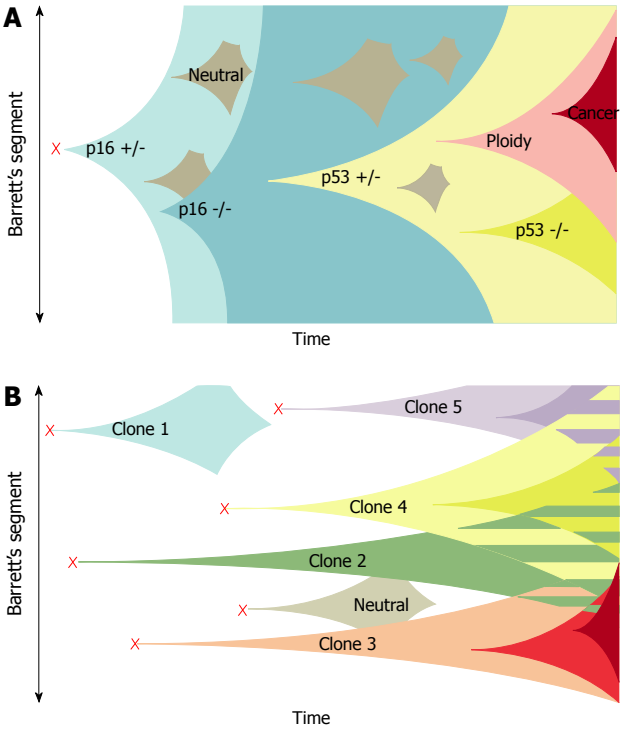

Figure 1 Clonal evolution models in Barrett's esophagus. A: The current model of clonal evolution adapted from Maley *et al*[66]. Founder mutation (red cross) occurs in a single progenitor and provides a growth advantage that predisposes to a selective sweep. Successive selective sweeps result in progression along the metaplasia dysplasia pathway. Clone bifurcation is responsible for the genetic heterogeneity in this model; B: The newly proposed model of evolution based on the mutation of multiple progenitor cells situated in esophageal gland squamous ducts located throughout the length of the esophagus (red crosses). Multiple independent clones then arise and evolve separately. The presence of multiple different clones gives rise to a mosaic interdigitating clonal pattern of the Barrett's segment represented as the striped areas[65].

Currently, dysplasia remains the only validated marker for identifying BE patients at risk, and forms the basis of EAC surveillance. However, this is imperfect. The tempo of progression towards EAC is highly variable and it remains unclear whether relentless progression through the MDC sequence is inevitable; some evidence suggests that high-grade dysplasia may remain stable for years or even regress[68]. Patients with BE may develop EAC during surveillance without detection of earlier MDC stages. This might relate to pace of progression, sampling error or lesions skipping directly from non-dysplastic disease to cancer. Other limitations of dysplasia as a prognostic marker include inter-observer variability in histological interpretation, and that inflammation may mimic dysplastic changes[69].

RISK FACTORS FOR NEOPLASTIC PROGRESSION

Until molecular biomarkers enter clinical practice it remains important to identify other clinical risk factors for malignant progression to effectively allocate resources and individualize surveillance programs, targeting those at highest risk. Identifying modifiable risk factors will also

Table 1 Published evidence from selected studies investigating genetic and epigenetic changes implicated in the metaplasia-dysplasia-carcinoma sequence of Barrett's esophagus

Factor	Summary of major findings/conclusions	Ref.
Growth self-sufficiency		
Cyclin D1	↑ nuclear cyclin D1 immunostaining in 46% BE specimens: -?cyclin D1 overexpression early event in MDC sequence	Arber *et al*[9]
	↑ nuclear cyclin D1 immunostaining in 64% EAC specimens	Arber *et al*[13]
	Cyclin D1 expression correlates with degree of dysplasia in BE	Coppola *et al*[14]
	Cyclin D1 expression 43% BE mucosa (*vs* 0% normal mucosa)	Umansky *et al*[15]
	Polyphenon E inhibits growth of BE and EAC cells *via* downregulation of cyclin D1 expression	Song *et al*[16]
Cyclin E	↑ cyclin E expression in neoplastic cells in BE	Coppola *et al*[14]
	Cyclin E expression 37% BE mucosa (*vs* 0% normal mucosa)	Umansky *et al*[15]
$p27^{Kip-1}$	83% EAC specimens displayed low p27 protein levels (despite high p27 mRNA): -p27 inactivated in most BE-associated EAC (post-transcriptional modification)→loss of cell cycle inhibition	Singh *et al*[17]
	Experimentally-induced BE and EAC development in mouse model significantly enhanced by p27 gene knockout	Ellis *et al*[18]
EGF (and EGF-R)	↑ EGF in cytoplasm of BE epithelial cells (*vs* gastric mucosa)	Jankowski *et al*[19]
	EGF-R expression area in inflamed mucosa (43.1%) significantly > normal mucosa (29.5%); all BE showed positive EGF-R staining	Jankowski *et al*[20]
	EGF/EGF-R expression significantly ↑ in BE and EAC mucosa (*vs* normal mucosa) by flow cytometry ($P < 0.01$)	Jankowski *et al*[21]
	EGF-R expression positive in 64% of BE-associated EAC; ↑ staining associated with poorer survival ($P = 0.004$)	Yacoub *et al*[22]
	EGF A61G G/G genotype associated with >double EAC risk in BE pts (*vs* A/A or A/G) (OR 2.2)	Lanuti *et al*[23]
TGF-α	↑ TGF-α expression in cells from BE and EAC mucosa (*vs* normal gastric mucosa) by flow cytometry ($P < 0.01$)	Jankowski *et al*[21]
	TGF-α expression positive in 100% of BE-associated EAC	Yacoub *et al*[22]
HGF (and HGF-R)	HGF expression significantly ↑ in BE specimens (*vs* normal esophageal mucosa)	Konturek *et al*[24]
	Intense HGF-R immunostaining in 100% EAC and dysplastic BE specimens (*vs* minimal staining in non-dysplastic BE or normal mucosa); HGF-R mRNA and protein levels ↑ in EAC cell lines	Herrera *et al*[25]
Erb family tyrosine kinases	Membranous c-erbB2 overexpressed in 26% EAC (*vs* 0% BE with dysplasia): -?later event in MDC sequence	Hardwick *et al*[26]
	c-erbB-2 gene amplification in 14% EAC *vs* 11% HG-dysplasia *vs* 0% metaplasia/LG-dysplasia specimens	Geddert *et al*[27]
FGF	Immunostaining intensity for FGF sequentially ↑ from metaplasia/LG-dysplasia (negligible)→HG-dysplasia (weak/moderate)→EAC (moderate/strong)	Soslow *et al*[28]
	FGF-1 mRNA and protein expression sequentially ↑ in HG-dysplasia/EAC (*vs* metaplasia/LG-dysplasia/controls)	Soslow *et al*[29]
Src family tyrosine kinases	Src-specific activity 3-4-fold ↑ in BE and 6-fold ↑ in EAC (*vs* controls): -?Src activation early event in MDC sequence	Kumble *et al*[30]
	Strong Src expression in 85% EAC *vs* 93% BE HG-dysplasia *vs* 72% BE LG-dysplasia *vs* 27% BE specimens	Iravani *et al*[31]
Insensitivity to anti-growth signals		
p16	9p21 (p16) LOH observed in 89% EAC specimens (*vs* 0% non-dysplastic BE); homozygous p16 deletion in only 25%	González *et al*[32]
	p16 promoter hypermethylation (inactivation) in 75% BE with HG-dysplasia *vs* 56% LG-dysplasia (*vs* 3% non-dysplastic BE)	Klump *et al*[8]
APC	5q (APC) LOH seen in 80% EAC specimens (and surrounding mucosa)	Barrett *et al*[33]
	APC gene LOH observed in 60% EAC specimens (*vs* 0% non-dysplastic BE)	González *et al*[32]
	APC promoter hypermethylation in 92% EAC *vs* 40% BE (*vs* 0% normal esophageal tissues)	Kawakami *et al*[34]
Avoidance of apoptosis		
p53	Positive p53 immunostaining in 87% EAC *vs* 55% BE with HG-dysplasia *vs* 9% LG-dysplasia *vs* 0% non-dysplastic BE	Younes *et al*[35]
	17p (p53) LOH found in 91% BE pts who developed aneuploid cell populations: -17p allelic losses precede aneuploidy	Blount *et al*[36]
	p53 overexpression in 64% EAC *vs* 31% dysplastic BE *vs* 0% non-dysplastic BE; trend of ↑ p53 expression with ↑tumour grade: -?p53 mutation early event in malignant progression	Symmans *et al*[37]
	p53 immunoreactivity only in EAC/BE with HG-dysplasia (not in BE with LG-/no dysplasia); mutated p53 in 69%: -?late event in MDC sequence (during transition to HG-dysplasia)	Rice *et al*[38]
	p53 protein expression in 85% EAC specimens *vs* 60% BE with HG-dysplasia *vs* 7% LG-dysplasia ($P < 0.001$)	Rioux-Leclercq *et al*[39]
	p53 mutations identified in 75% EAC specimens; p53 overexpression in 58% EAC *vs* 60% BE with HG-dysplasia *vs* 12% LG-dysplasia *vs* 0% non-dysplastic BE	Chung *et al*[40]
Fas (CD95)	↓ surface expression of Fas observed in EAC specimens; impaired translocation of Fas to membrane wild-type Fas protein retained in cytoplasm in EAC cell line: -?potential mechanism by which EAC cells evade Fas-mediated apoptosis	Hughes *et al*[41]
	↓ surface expression of Fas and resistance to Fas-mediated apoptosis observed in EAC cell lines	Mahidhara *et al*[42]
Bcl-xl/Bax/Bcl-2	Bcl-xl positive in all dysplasia and EAC cells, but negative in 47% non-dysplastic BE: -?switch to anti-apoptotic phenotype in transformation from metaplasia to EAC	van der Woude *et al*[43]

	Bcl-2 expression in 84% LG-dysplasia *vs* 0% HG-dysplasia or EAC	Rioux-Leclercq *et al*[39]
	Cytoplasmic Bcl-xl immunostaining in 59% EAC *vs* 71% BE/HG-dysplasia *vs* 60% LG-dysplasia *vs* 27% non-dysplastic	Iravani *et al*[31]
COX-2	↑ COX-2 mRNA levels in 80% BE and 100% EAC specimens (*vs* normal gastric controls) ($P < 0.001$); COX-2 immunostaining strongly positive in 100% BE samples (> gastric controls)	Wilson *et al*[44]
	COX-2 immunopositivity in 91% non-dysplastic BE *vs* 94% dysplastic *vs* 97% EAC	Lagorce *et al*[45]
	Natural/synthetic COX-2 inhibitors suppressed proliferation, induced apoptosis and blocked cell cycle in EAC cell lines	Cheong *et al*[46]
	Cox-2 mRNA strongly upregulated in experimentally-induced BE epithelium in rat model (*vs* absent in control animals); COX-2 overexpression observed in human BE patients with dysplasia	Majka *et al*[47]
Limitless replicative potential		
Telomerase	Telomerase RNA positive in 100% EAC/BE with HG-dysplasia *vs* 90% LG-dysplasia *vs* 70% non-dysplastic BE: marked ↑ telomerase RNA accompanies transition along MDC sequence	Morales *et al*[48]
	human telomerase reverse transcriptase (catalytic subunit of telomerase) expression ↑ at all stages of BE *vs* normal controls, and in EAC ($P = 0.003$) and dysplastic BE ($P = 0.056$) *vs* non-dysplastic BE	Lord *et al*[49]
	Telomerase activity (by telomeric repeat amplification protocol assay) ↑ in EAC samples *vs* adjacent mucosa ($P = 0.0002$) and in EAC *vs* BE ($P = 0.001$); no difference BE *vs* adjacent mucosa	Barclay *et al*[50]
	Telomerase inhibition (by small interference RNAs) induced senescence in 40% and apoptosis in 86% in BE cell lines	Shammas *et al*[51]
Sustained angiogenesis		
VEGF (and VEGF-R)	VEGF expression correlated with higher vascularisation in BE and EAC specimens	Couvelard *et al*[52]
	VEGF-A expressed in BE epithelium; VEGFR-2 strongly expressed in immature endothelial cells feeding BE epithelium; ↑ VEGF-C expression in BE (*vs* absent in normal epithelium); ↑ VEGFR-3 in EAC: ?aberrant neovasculature early in MDC sequence	Auvinen *et al*[53]
	VEGF expressed in 64% EAC specimens; significantly correlated with angiolymphatic invasion/survival	Saad *et al*[54]
	VEGF expression significantly ↑ in EAC (> dysplastic BE > BE > normal epithelium)	Griffiths *et al*[55]
Invasive/metastatic potential		
CAMs	↓ expression in EAC specimens of E-cadherin (in 74%), α-catenin (60%) and β-catenin (72%)	Krishnadath *et al*[56]
	Abnormal expression of β-catenin ($P = 0.022$), α-catenin ($P < 0.01$) and E-cadherin ($P = 0.049$) significantly associated with higher degrees of BE-related dysplasia	Washington *et al*[57]
	↓ expression of E-cadherin with progression along MDC sequence ($P < 0.01$); in contrast P-cadherin absent from BE (± dysplasia) but expressed in 67% EAC specimens	Bailey *et al*[58]
	Slug (E-cadherin repressor) immunostaining and mRNA levels overexpressed in EAC *vs* BE metaplasia specimens: -?Slug upregulation represents mechanism of E-cadherin silencing	Jethwa *et al*[59]
Cathepsins	Detected amplicon at chromosome 8p22-23 resulting in cathepsin B overexpression (observed in 73% EAC samples)	Hughes *et al*[60]
	↑ cathepsin C expression in EAC (*vs* BE *vs* normal) in rat model	Cheng *et al*[61]
CD44	Stepwise ↑ cathepsin D mRNA levels in GERD→BE→EAC tissue	Breton *et al*[62]
	CD44-H and -V6 variant frequently expressed in BE; differing expression patterns along spectrum normal→dysplastic BE→EAC: -?CD44H and V6 involved in carcinogenesis of BE mucosa	Lagorce-Pages *et al*[63]
	↓ CD44 expression in EAC/HG-dysplasia (*vs* BE/LG-dysplasia)	Darlavoix *et al*[64]

BE: Barrett's esophagus; MDC: Metaplasia-dysplasia-carcinoma; EAC: Esophageal adenocarcinoma; EGF: Epidermal growth factor; EGF-R: EGF receptor; pts: Patients; OR: Odds ratio; TGF: Transforming growth factor; HGF: Hepatocyte growth factor; HGF-R: HGF receptor; mRNA: Messenger RNA; FGF: Fibroblast growth factor; HG: High grade; LG: Low grade; LOH: Loss of heterozygosity; APC: Adenomatous polyposis coli; COX-2: Cyclooxygenase-2; VEGF: Vascular endothelial growth factor; VEGF-R: VEGF receptor; CAM: Cell adhesion molecule; GERD: Gastro-esophageal reflux disease.

Table 2 Clinical and demographic risk factors for neoplastic progression of Barrett's esophagus

Innate factors	Gastrointestinal factors	Other modifiable factors
Age	Bile and acid reflux	Obesity
Gender	Anti-reflux surgery	Diet
Ethnicity	Proton pump inhibition	Alcohol
	Pharmacological lower esophageal sphincter relaxation	Smoking
	Salivary nitrates	Socioeconomic status
	Barrett's segment length	Pharmacological COX-2 inhibition

COX-2: Cyclooxygenase-2.

inform disease prevention strategies. Epidemiological studies of EAC have described a "birth cohort effect", with higher incidence rates observed in recent cohorts suggesting exposure to an exogenous risk factor in early life contributing increased risk in all ages of the cohort[70] (Figure 2). Multiple risk factors for neoplastic progression of BE have been investigated (Table 2).

INNATE HOST FACTORS

Age is a well-recognized risk for both BE and EAC. Corley *et al*[71] reported an incidence of BE of 2/100 000 for 21-30-year-old and 31/100 000 for 61-70-year-old, whilst El-Serag *et al*[70] calculated the risk of EAC to increase by 6.6% for each 5-year age increase. Evidence specifically linking age to risk of neoplastic progression within BE is lacking, but it seems intuitive to propose advancing age as an independent risk factor.

BE displays a male preponderance of approximately 2:1, rising to 4:1 for BE-associated EAC, suggesting an independent influence of gender on risk of neoplastic pro-

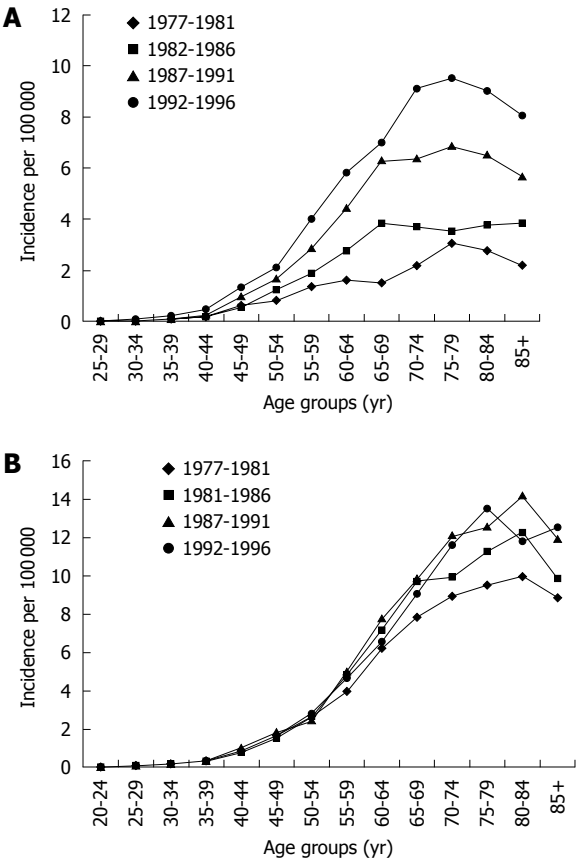

Figure 2 Age distribution of cases diagnosed with oesophageal adenocarcinoma (A) and gastric cardia adenocarcinoma (B) in the USA between 1977-1996, displaying the "birth cohort effect". Each individual curve represents the age-specific incidence rates in a five year period (from El-Serag *et al*[70]).

gression[71,72]. Why male gender should confer additional risk is unknown; some have speculated that male propensity toward visceral pattern of obesity might be relevant[73].

A higher prevalence of BE in Caucasians has long been recognized[74]; again, this association strengthens with development of BE-associated EAC[75]. Analysis of the US Surveillance, Epidemiology and End Results registry found that the annual incidence of EAC for Caucasian males was double that for Hispanic males and four times higher than Black, Asian, Pacific Island and Native American males[76]. Although selection bias and differing endoscopy uptakes between ethnic groups might partially explain this, other factors seem to be involved. Whilst environmental influences are probably important, hitherto-unidentified genetic variations influencing protection against reflux-induced mucosal damage seem likely. A US study found similar GERD prevalence in Caucasian and Black Americans from the same geographical population, yet the latter displayed significantly less esophagitis and almost no BE[77].

GASTROINTESTINAL FACTORS

Bile/acid reflux

The relationship between GERD and BE is well established, and whilst reflux of gastric acid is known to induce chronic mucosal esophageal injury the contribution of bile salts and acids (from duodenal refluxate) is increasingly recognized. Vaezi and Richter demonstrated patients with complicated BE (dysplasia/stricture/ulceration) reflux significantly greater amounts of both gastric and bile acids than those with uncomplicated BE, and postulated that complications might result from synergism between the two[78]. Bile salts induce esophageal injury over a wide pH range, and patients with BE display significantly more bile salts in aspiration studies than patients with mild reflux only[79]. Menges *et al*[80] observed a strong correlation between duration of esophageal exposure to acid and bile with severity of pathological change in BE. Furthermore, proton pump inhibitor (PPI) therapy predisposes to upper gastrointestinal bacterial colonization and consequent bile salt-deconjugation, which, in this high pH environment, has been linked to chronic inflammation[81].

Refluxate-mediated inflammation might promote carcinogenesis *via* both the arachidonic acid (AA) pathway and induction of oxidative stress. Low pH and bile salts promote expression of cyclooxygenase-2 (COX-2), catalyzing conversion of AA into various prostaglandins, including PGE_2. PGE_2 increases proliferation of BE epithelial cells and inhibits tumor surveillance through suppressing natural killer cell function. Consequently, abnormal cells displaying genomic instability may accumulate. COX-2 expression has been shown to increase with neoplastic progression of BE, supporting a role for the AA pathway in EAC carcinogenesis[44]. Chronic mucosal injury also induces production of reactive oxygen species (ROS), depletes antioxidants and increases expression of oxidative stress-related genes. High levels of oxygen radicals and lipid peroxidation products have been demonstrated in BE epithelial cells, with reduced levels of vitamin C and glutathione, indicating compromised oxidant defences[82]. ROS have well-established mutagenic capacity, whilst subsequent apoptosis of mutated cells is additionally suppressed by capacity of bile salts to induce proteasomal degradation of *p53*[83].

The Factors Influencing the Barrett's Adenocarcinoma Relationship (FINBAR) study suggested GERD symptom chronicity and frequency appeared better predictors for neoplastic progression than severity[84]. However, a significant proportion of EAC patients (40%-50%) do not recall ever having prior reflux[85]. Furthermore, reflux of gastroduodenal contents correlates poorly with heartburn symptoms, BE is frequently asymptomatic and development of less sensitive Barrett's epithelium may ameliorate symptoms. Thus, symptom-based risk scores for assessing progression risk have so far not proved useful in clinical practice.

PPIs

PPIs increase pH of gastric refluxate, attenuating acid-induced damage. Ouatu-Lascar *et al*[86] showed "normalization" of intraesophageal pH with acid suppression favors differentiation and reduces cellular proliferation in BE biopsy specimens. However, PPIs have not prevented

recent increases in EAC, and the observation of EAC with PPI administration in animal models raises concern they might actually favor progression of BE[87]. This might be mediated *via* interaction of gastrin with its cholecystokinin receptor, CCK_2R. PPIs elevate serum gastrin levels, which on binding to CCK_2R, stimulate expression of EGF and trefoil peptide, inducing COX-2 expression. Gastrin exposure increases proliferation in esophageal cell culture, and BE mucosa expresses more CCK_2R than normal squamous mucosa. CCK_2R stimulation also inactivates pro-apoptotic factors[88].

Despite this, the clinical relevance in humans remains unproven. Three large studies have examined PPI usage and EAC risk in BE patients, each reporting a strong inverse correlation. Two observed a decreased risk with longer duration of PPI, and one showed an increased risk with delayed PPI use[89]. Obszynska *et al*[90] investigated effects of hypergastrinemia induced by different PPI doses in cell models and BE patients. Despite increased cell proliferation *in vitro*, COX2 induction and enhanced epithelial restitution, they found no evidence of longer-term harm using surrogate biomarkers of proliferation or apoptosis *in vivo*. The Aspirin Esomeprazole Chemoprevention Trial (AspECT) is currently investigating effects of different PPI doses in combination with aspirin on EAC risk.

Anti-reflux surgery

Theoretically, anti-reflux surgery should prevent reflux of duodenal contents, against which PPIs have no effect, potentially mitigating against progression of BE. Unfortunately this is not supported by the available evidence. Two large cohort studies failed to show cancer protection in GERD patients[91,92], whilst a meta-analysis by Corey *et al*[93] concluded no reduction in progression risk for BE. However, different surgical procedures were employed and effectiveness of reflux control was not always assessed.

Lower esophageal sphincter-relaxing drugs

Pharmacological lower esophageal sphincter (LES) relaxation might promote development/progression of BE by increasing reflux, suggested by the observation that drugs with these effects (e.g. tricyclic antidepressants) have increased in use alongside the rise in EAC. A Swedish population-based study by Lagergren *et al*[94] reported a positive association between EAC and long-term use of LES-relaxing drugs, with the strongest association for anticholinergics; this association disappeared after adjustment for reflux symptoms.

Helicobacter pylori infection

An increase in BE-associated EAC alongside falling rates of *Helicobacter pylori* (*H. pylori*) infection has led some to propose a protective effect of *H. pylori*, mediated by its influence in reducing gastric acidity. The virulent cagA strain is particularly associated with high-grade gastric inflammation and atrophy[95]. A meta-analysis by Rokkas *et al*[96] reported statistically significant inverse relationships between *H. pylori* infection and both EAC and BE [odds ratio (OR), 0.52% and 0.64%, respectively]. Furthermore, a large prospective study of BE patients and GERD controls found less *H. pylori* infection with increasing "severity" of disease: 44% in GERD; 35% in uncomplicated BE; 14%-15% in BE with high-grade dysplasia/EAC[97].

However, another study, controlling for demographic and lifestyle factors, failed to demonstrate reduced EAC with cagA+ infection[98]. A confounding factor might be the degree of bile acid reflux, since excessive bile reflux may prevent *H. pylori* colonization and contribute to chronic mucosal injury[88]. The protective role for *H. pylori* is debatable and since *H. pylori* is a World Health Orgaisation class 1 mutagen for gastric adenocarcinoma it is difficult to argue against its eradication whenever it is detected.

Salivary nitrates

Dietary nitrate, concentrated in saliva and reduced to nitrites by oral flora, produces intraesophageal nitric oxide (NO) during reflux. Achlorhydria induced by PPI or atrophic gastritis may cause overgrowth of nitrate-reducing bacteria in the upper gut, providing another source of nitrite[88]. Clemons demonstrated the capacity of NO to induce double-strand DNA breaks in esophageal BE cells *in vitro*, which could promote neoplastic progression[99]. Increasing agricultural nitrate use in the latter 20th century caused significant increases in nitrate content of leafy vegetables and drinking water[100] and could have partially contributed to the increase in EAC incidence.

Barrett's segment length

Although EAC can develop in BE segments of any length, several observational studies support the intuitive notion that longer segments confer greater risk[101]. However, a meta-analysis by Thomas *et al*[102] showed only a non-significant trend towards reduced progression with shorter BE segments, and evidence remains insufficient to advocate surveillance strategies based on segment length alone.

OTHER MODIFIABLE RISK FACTORS

Obesity

Increasing obesity has also paralleled increased rates of BE and EAC. Strong links between obesity and both GERD and erosive esophagitis have been established[103]. It is logical that this might predispose to BE, but a meta-analysis specifically comparing body mass index (BMI) in BE cases with population controls showed only a modest risk increase[104]. However, elevated BMI is a strong risk factor for EAC (OR, 1.8 and 2.4 for BMI > 25 and BMI > 30, respectively)[105]. Increased risk may relate more to distribution of body fat than BMI alone, with visceral (abdominal) obesity conferring greater risk[106]. Other studies noted an association between obesity in early life and EAC risk, suggesting adiposity may act early in the disease process[84,107].

Although a small prospective study by Oberg and colleagues failed to identify any association between BMI

Table 3 Selected published evidence linking adipokines (and ghrelin) with Barrett's esophagus and progression to esophageal adenocarcinoma

Adipokine	Evidence in BE and EAC	
	Relevant study findings	Ref.
Adiponectin	↓ adiponectin receptors in Barrett's mucosa compared with normal mucosa from controls	Konturek *et al*[110]
(↓ in obesity)	↑ Bax (pro-apoptotic), ↓ Bcl-2 (anti-apoptotic) and ↑ apoptosis of EAC cell lines on incubation with adiponectin	Konturek *et al*[110]
	Plasma adiponectin levels inversely associated with BE risk in 50 matched cases (OR 4.7 for each 10 μg/mL ↓ in level) (independent of BMI)	Rubenstein *et al*[111]
	No difference in adiponectin levels between 51 BE patients and 67 controls	Kendall *et al*[112]
Leptin	Leptin receptors expressed in esophagus	Francois *et al*[113]
(↑ in obesity)	↑ proliferation and ↓ apoptosis (*via* various signalling pathways) in EAC cell lines	Ogunwobi *et al*[114]
	Leptin levels strongly associated with ↑ risk of BE in males (no association in females)	Kendall *et al*[112]
	Gastric (fundic) leptin levels positively associated with BE (no association with serum leptin)	Francois *et al*[113]
Ghrelin	↑ gastric emptying (so may ↓ gastric reflux)	Dornonville *et al*[115]
(↓ in obesity)	↓ TNF-α-induced COX-2 and interleukin-1-β expression in BE cell line	Konturek *et al*[110]
	Ghrelin expression negligible in archived EAC cell specimens (*vs* rich expression in normal mucosa)	Mottershead *et al*[116]
	↑ serum ghrelin associated with ↓ EAC risk (in overweight subjects)	de Martel *et al*[117]

BE: Barrett's esophagus; EAC: Esophageal adenocarcinoma; OR: Odds ratio; BMI: Body mass index; COX-2: Cyclooxygenase-2; TNF: Tumor necrosis factor.

and progression from BE to low- or high-grade dysplasia[108], it had limited power, and a larger study from the Seattle Barrett's Esophagus Program revealed strong correlations between waist-to-hip ratio and intermediate biomarkers of progression[109]; again, associations were less apparent for elevated BMI *per se*.

Obesity causes GERD through several mechanical and physiological mechanisms. However, part of the association between obesity and EAC is independent of GERD, suggesting a role for reflux-independent mechanisms, probably linked to important endocrine actions of adipose tissue. Many recent studies have linked several adipokines (metabolically active factors) to plausible actions in the MDC process[110-117] (Table 3).

Kristal *et al*[118] investigated whether weight loss (alongside other dietary measures) impacted upon measured biomarkers of cellular proliferation in BE. Despite weight loss (mean 3.6 kg) at 18 mo no differences in biomarkers were observed. This study was relatively small, and the lack of response might relate to the relatively modest weight loss, and/or choice of proliferation markers employed.

Diet

Several studies have shown an association between a diet high in fruit and vegetables and reduced EAC. A large population-based Swedish study found individuals in the highest exposure quartile of fruit and vegetable intake to have approximately 50% less EAC compared to the lowest quartile[119]. However, Kristal *et al*'s study observed no effect on biomarkers of BE cell proliferation despite a net increase in fruit and vegetable consumption[118], whilst the FINBAR study observed a reduction in EAC with increased fruit, but not vegetable, consumption[84]. A protective effect for the natural anti-oxidants in fruit was proposed. A well-controlled, prospective study by Dong *et al*[120] showed patients who took multivitamin pills had significantly decreased risk of tetraploidy [hazard ratio (HR), 0.19] and frank EAC (HR, 0.38). Significant inverse associations with EAC were also observed for supplemental vitamins C (HR, 0.25) and E (HR, 0.25), both well-recognized antioxidants.

Chen *et al*[121] observed a significant inverse association between zinc intake and EAC risk compared with controls (OR, 0.5); inverse associations were also noted for vitamin A, β-cryptoxanthin, riboflavin, folate, fiber, protein and carbohydrate, whilst saturated fat intake was positively associated with EAC. Rudolph *et al*[122] investigated selenium levels in 396 BE patients: those with levels in the upper three quartiles were less likely to display high-grade dysplasia (OR, 0.5), aneuploidy (OR, 0.4) or 17p LOH (OR, 0.5) than the lowest quartile. No association was observed with *p16* LOH (an early event in the MDC sequence), indicating selenium's protective effects might occur late in progression to EAC.

Alcohol

Data supporting links between alcohol and BE/EAC are sparse. The UK BE registry found no association between alcohol consumption in patients with BE compared with reflux esophagitis[123]. Although at least eleven studies have investigated the relationship between alcohol and EAC only six have shown a positive association, and in most it was weak[124-134]. One study even seemed to suggest wine to be protective[133].

Smoking

Studies of smoking and BE/EAC are contradictory. An Australian population-based case-control study found smoking was associated with 2- to 3-fold increased risk of BE and BE with dysplasia[135]. However, there was no dose-response effect. Other small studies found no clear association[131]. Whilst smoking is a strong risk factor for esophageal squamous cell carcinoma, studies of EAC have been inconsistent, yielding conclusions ranging from complete absence of association[132-134] to a significant OR of 3.4 for current smokers[128]. Problems with study methodology occur and certainly smoking has rarely been a primary endpoint for studies of BE/EAC.

Socioeconomic status

There are no clear associations between socioeconomic status and neoplastic progression of BE. Some studies suggest increased EAC risk in higher socioeconomic groups, others the reverse[72].

COX-2 inhibition

Given the role of the AA pathway in neoplastic progression, pharmacological inhibition of COX-2 might modify the natural history of BE. Various studies have investigated whether aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) might confer protection against EAC. A meta-analysis by Corley *et al*[136] including 1813 EAC patients suggested a protective association (OR, 0.67). Both intermittent and frequent use appeared advantageous, with evidence of a dose-effect, whilst aspirin conferred greater protection than NSAIDs.

However the Chemoprevention for Barrett's Oesophagus Trial randomized 100 BE patients with dysplasia to either celecoxib 200 mg twice daily or placebo, with negative results[137]. A retrospective analysis of the UK BE registry with a total follow-up of 3683 patient-years also failed to demonstrate a protective effect of aspirin[138]. AspECT should provide further useful information.

CONCLUSION

The etiology of progression of BE is probably multi-factorial, with contributions from environmental risk factors interacting with genetically-determined characteristics. Obesity and ongoing bile and acid reflux are emerging as potentially modifiable risk factors, though designing practical interventions has so far proved difficult. Developments in understanding the MDC process in BE may provide future testable therapeutic targets.

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