The role of inflammation in cancer of the esophagus


Abstract

Esophageal adenocarcinoma is the eighth most common malignancy worldwide. The overall prognosis is poor, with 5-year survival rates of approximately 15–25%, and 30–50% for patients who can be treated with curative intent. There has been a marked increase in incidence of esophageal adenocarcinoma over the last 30 years, with chronic and severe reflux, diet and obesity identified as principal factors fuelling this rise in the West. Esophageal adenocarcinoma is an exemplar model of an inflammation-associated cancer. The key molecular pathways driving tumor development and influencing tumor biology are the subject of considerable research efforts, and is the principal focus of this review. In addition, the diverse range of changes occurring in the local immune response, tissue microenvironment, metabolic profile, intracellular signaling mechanisms and microRNA signatures are discussed, as well as novel targeted therapies.

The incidence of esophageal adenocarcinoma (EAC) has risen rapidly over the past three decades, particularly in the West [1]. More recently, a report of EAC incidence from the Surveillance, Epidemiology and End Results program (SEER) confirms a continued rise from 13.4 per million in 1973 to 51.4 per million in 2009. This represents an almost fourfold increase, suggesting that EAC in the USA has increased more than any other malignancy and that the observed increase is true, sustained and not an artifact of surveillance [2]. Epidemiologically, the rise parallels the increasing prevalence of gastroesophageal reflux disease (GERD), Barrett’s esophagus (BE) and obesity in affected societies [3]. EAC is currently the sixth leading cause of cancer death worldwide [4]. The overall prognosis is poor, with 1-5 year survival ranging from 15 to 25% and between 30 and 50% in patients that can be treated with curative intent [5].

EAC has been described as an ‘exemplar model’ of inflammation-associated cancer [6]. BE, characterized by specialized intestinal metaplasia (SIM), is the sole recognized pathologic precursor of EAC. It is associated with an annual risk of progression of between 0.12 and 0.25% (REF). GERD, particularly severe or chronic GERD, may predispose to BE and is also independently associated with risk of EAC [6]. In BE, targeting inflammation through the use of aspirin and other anti-inflammatory drugs has shown promise. Aspirin is being studied in the large APECT trial, which is underway at present [7,8]. The emerging consensus is that multiple proinflammatory pathways fueled by GERD, BE and obesity are important to the pathogenesis of EAC. Furthermore, an improved understanding of the key pathways linking inflammation and esophageal carcinogenesis may provide therapeutic targets. This review focuses on current understanding of these molecular pathways by first describing the factors initiating the inflammatory response, specifically bile reflux and obesity. We then discuss the tumor microenvironment and move sequentially deeper into the cell microstructure, discussing the impact of transcriptional regulation, mitochondrial alterations and miRNAs and genomic instability within the nucleus in the context of inflammation.

Inflammation & cancer

The idea that tumors behave like a ‘never healing wound’ is not novel [9]. Virchow in 1863 observed the presence of leukocytes in malignant tissue and first linked cancer with a state of chronic inflammation [10]. Increased understanding of the inflammatory microenvironment has produced evidence supportive of the role of inflammation in tumorigenesis. As
such, ‘inflammation’ is now accepted as the seventh hallmark of cancer complementing the existing six – the ability of cancer cells to proliferate, resistance to inhibitory signals and apoptosis, angiogenesis, immortality and the ability to metastasize [13]. Wound healing and tumor stroma formation exhibit many similarities, supporting Virchow’s postulate [11]. In response to tissue damage, a multifactorial network of cellular signals raises a host response designed to infiltrate the area with immune cells and ‘heal’ the wound. The difference in tumor tissue is that unlike wound healing, tumors lead to a persistent state of extracellular matrix (ECM) generation. The ECM serves to form a scaffold allowing fibroblasts and endothelial cells to proliferate and migrate. The persistence of acute inflammatory initiating factors results in a chronic state of inflammation. Host leucocytes infiltrate both the stroma and tumor itself [4]. Tumor-associated macrophages exhibit duality of function, with classically activated macrophages retaining the ability to kill neoplastic cells following activation by IL-2, IFN-γ and IL-12, whereas alternatively activated macrophages also infiltrate tumors and produce growth and angiogenic factors as well as initiating ECM degradation [15]. This leads to proliferation, angiogenesis and metastasis. Tumor cells exhibit the capacity to produce a network of inflammatory cytokines and chemokines, which contribute to malignant progression [16]. Epidemiological studies conducted in recent years have demonstrated a strong link between inflammatory responses and the development of cancer [17].

Acid/bile reflux induces esophageal inflammation

The first reports of esophageal inflammatory injury being caused by gastric reflux were made by Quincke at the end of the 19th century, and further by Winkelstein in 1935 [18]. Reflux is enabled by deficient lower esophageal sphincter (LES) pressure; this may be evident in association with a hiatus hernia. In recent times, transient LES relaxations (TLESRs) Figure 1 are accepted as important pathophysiological abnormalities seen in patients with GERD. Refluxate may be acid alone commonly, occasionally bile alone or a combination of acid and bile, this latter combination being common in BE. Bile in the esophagus is linked to less effective esophageal motility [19,20]. Salts of taurocholic and glycocholic acid represent approximately 80% of bile salts, which are conjugated versions of bile acid [21]. The pKa of each acid is 7. It has been demonstrated in vivo that bile acid concentrations are higher in the esophageal aspirates of patients with GERD and BE compared with controls and that mixed acid/bile reflux is more harmful than acid reflux alone [22]. Animal models have demonstrated that
esophageal bile exposure via either direct perfusion or total gastrectomy with esophageojejunal anastomosis results in severe esophagitis, Barrett's metaplasia and EAC [23]. Reflux of bile acids in concentrations greater than 200 micromolar has been demonstrated in 50% of patients with severe esophagitis and BE, and there is a synergistic effect resulting from the presence of bile with low concentrations of bile acids, with epithelial damage seen only at high acid levels [21]. Activation of the IL-6/STAT-3 anti-apoptotic pathway following exposure to bile acids has been cited as a potential explanation of the development of dysplasia and tumor progression [24].

In patients with GERD, chronic inflammation is the result of continued acid exposure, which may also be combined with bile refluxate. Although healing is possible via regeneration of squamous cells, it also occurs via replacement of squamous with columnar cells and SIM, resulting in BE [25]. This persistent state of chronic inflammation gives rise to release of proinflammatory mediators, which promote cell growth and invasion, thus supporting the transformation and initiation of tumor development [26]. Key cytokines implicated in this process to date include IL-8, IL-6, TGF-β and IL-1β [27–29]. Although further work is required to understand the exact mechanism by which the stromal compartment can induce cancer in patients with BE, it is increasingly likely to be playing a role in the recruitment of inflammatory cells in response to reflux injury.

Obesity is a proinflammatory state

A recent meta-analysis by Singh et al. has confirmed the association between central adiposity, independent of body mass index, and esophageal inflammation, metaplasia and neoplasia [30]. The authors concluded that the effects were mediated by both reflux-dependent and -independent mechanisms [30]. Two main hypotheses have been utilized to explain this association to date. First, the association between increased BMI and GERD, and consequently, BE is well established [31]. This has long been attributed to the mechanical hypothesis, whereby the increased intragastric pressure resulting from abdominal obesity disrupts the structure and function of the LES and is permissive to reflux. However, studies suggest that obesity does not per se cause increased acid exposure, and consequently, there is an emerging focus on non-mechanical influences driving this association [32].

Obesity represents a chronic state of low-grade inflammation characterized by increased storage of fatty acids in an expanded tissue mass [33]. Obese children and adults have increased plasma levels of C-reactive protein, IL-6, TNF-α and leptin [34]. An increase in the levels of non-esterified fatty acids resulting from an inability of existing adipose tissue to buffer excess nutrient intake is typical of the metabolic syndrome [35]. Higher lipolytic activity in visceral adipocytes allows more rapid mobilization of free fatty acids, which are reflected in the systemic circulation of obese individuals [36,37]. Excess adipose tissue results in an increase in proinflammatory cytokines, leading to systemic low-grade inflammation [38]. Insulin resistance and Type 2 diabetes mellitus are also associated with the presence of systemic inflammation. Nutritionally induced insulin resistance is the result of adaptation to the ongoing release of circulating free fatty acids, resistin and TNF-α, which are released from visceral fat stores in addition to decreased release of adiponectin [38]. The result is reduced responsiveness in muscle, liver and adipose tissues to insulin, as well as compensatory hyperinsulinemia [39]. The state of hyperinsulinemia results in downregulation of insulin receptor levels and also dampened responsiveness of intracellular signaling pathways mediating its effects [40]. Insulin itself has been shown to exhibit tumorogenic effects in a number of tissue types, which are mediated by insulin receptors in the preneoplastic target cells, or changes in endogenous hormone metabolism secondary to hyperinsulinemia [38–41,43]. Intracellular signaling pathways hypothesized to link inflammation and insulin resistance include JNK and IκB kinase. Genetic or chemical inhibition of these pathways can improve insulin resistance [44,45]. Systemic markers for oxidative stress also increase with adiposity, consistent with the role of reactive oxygen species (ROS) in the development of obesity-induced insulin resistance [46]. The accumulation of lipids and resultant activation of nicotinamide adenine dinucleotide phosphate-oxidase increases ROS production, which in turn, increases TNF-α, IL-6 and MCP-1 production and decreases adiponectin level [38]. Among patients with BE, increased levels of leptin and insulin resistance are associated with an increased risk of EAC, while adiponectin levels demonstrate a negative association [47–50].

Proinflammatory cytokines contribute to the initiation & promotion of tumor growth

In BE, the maximal degree of inflammation characterized by cytokines such as IL-1β is centered in the squamous mucosa adjacent to the tumor. An inflammatory gradient is reported, with molecular inflammation reduced distally and characterized by a significant increase in anti-inflammatory IL-10 expression [51]. In a transgenic mouse model of BE, esophageal overexpression of IL-1β phenocopies human pathology with the evolution of esophagitis, Barrett-like metaplasia and EAC, suggesting that tumor-promoting IL-1β and also upregulation of IL-6 signaling are important in the BE/EAC paradigm [52]. Moreover, IL-8 and IL-1β are markedly elevated in biopsy specimens of esophagitis and BE, with further increases observed in EAC [53]. The source of cytokines may be not only infiltrating inflammatory cells. Barrett’s epithelial cells are capable of expressing IL-6 and IL-1β. Also, bile acids, in particular deoxycholic acid, is capable of inducing both via activation of NF-κB [53]. TNF-α is upregulated in the BE/EAC progression and induces the oncogene c-myc expression via beta-catenin-mediated transcription independent of NF-κB [54]. IL-6 and IL-6 mRNA expression are increased in transformed Barrett’s cell lines compared with non-transformed lines along with STAT3 [55].

TGF-β1 has anti-inflammatory and tumor-suppressive properties under normal conditions; however it is linked with tumorigenesis in an abnormal microenvironment [56]. In a series of resected Barrett’s adenocarcinoma of the distal esophagus, relative expression of the TGF-β1 gene was significantly higher in tumor tissue compared with squamous epithelium and Barrett’s mucosa [57]. It was also found to be associated with advanced-stage, nodal
involvement and lymphatic vessel invasion with overexpression associated with a negative impact on survival [57]. TGF-β1 signaling is initiated by activation of type I and II transmembrane serine/threonine kinase receptors (TβRI and TβRII), leading to phosphorylation of the intracellular signaling molecules Smad2 and Smad3 [58]. This complex is associated with Smad4. When translocated to the nucleus, the result is transcriptional regulation of a number of target genes including c-Myc- and cyclin-dependent kinase inhibitors, allowing it to potentially function as a negative growth factor [59]. TGF-β1 responsiveness is reduced during all stages of the Barrett’s-metaplasia-dysplasia sequence due to abnormalities at several points in the signaling pathway, but primarily Smad4 [59].

The epidemiologic link between the use of NSAIDs and reduced incidence of esophageal cancer has prompted investigation into the inflammation and carcinogenesis-associated expression of cyclooxygenase-2 (COX-2) [60,61]. Bile acid exposure results in substantial up-regulation of COX-2 in esophageal tissue and COX-2 expression is significantly higher in patients with SIM, dysplasia and adenocarcinoma compared with normal squamous epithelium [61]. As one of the two isoforms of the COX enzyme, it is thought to be involved in resisting apoptosis, increasing cell proliferation, stimulating angiogenesis and modulating the invasive properties of cancer cells, and COX-2 is associated with increased lymph-node metastases and reduced survival in Barrett’s-associated EAC [62].

**Inflammation & immune modulation of the tumor microenvironment**

Chronic inflammation results in a microenvironment conducive to neoplastic change. Inflammatory immune cells including neutrophils, macrophages, dendritic cells and lymphocytes infiltrate the site of inflammation Figure 2. Myeloid and plasmacytoid dendritic cells are recruited during the metaplasia to carcinoma sequence in the esophagus [63]. In vivo, myeloid dendritic cells co-cultured with BE and EAC cells stimulate regulatory T cell differentiation from naïve CD4+ T cells promoting tumor progression [63]. Metastatic cells in BE either as a consequence of TGF-α or of TNF-α stimulation secrete VEGF, which can promote adjacent endothelial cell growth via phosphorylation of beta-catenin and vascular endothelial cadherin [64]. An additional source of VEGF is macrophages. While present in similar numbers in reflux esophagitis and BE, macrophages are increased in EAC and also produce matrix metalloproteinases (MMP)-12, which increases across the BE to EAC spectrum [65]. This may result in acceleration of angiogenesis and increased microvascular invasion. Inflammation at the site of
metaplasia is characterized by an increase predominantly in Th2 effector cells, and also Th1 effector cells in comparison with reflux esophagitis, supporting the hypothesis that specific esophageal immune responses may influence disease progression [66]. Infiltration of eosinophils has also been demonstrated in the mucosa of a subset of BE patients associated with basal cell hyperplasia [66].

The ECM is modified to support the infiltration of immune cells. Matricellular proteins represent a unique group of proteins, and secreted protein acidic and rich in cysteine is increased in both BE and EAC. Secreted protein acidic and rich in cysteine demonstrates counter-adhesive and anti-proliferative functions and can modify the cell cycle and remodel matrix [67]. It is thought to be overexpressed in the tumor microenvironment in an attempt to inhibit tumor growth [7]. Thrombospontin-1 is another matricellular protein with angiogenic effects and the capacity to regulate TGF-β1, it is differentially up-regulated across the EAC sequence [7]. Matrix metalloproteinases are a family of protein-degrading enzymes, which function as endopeptidases. There are currently 25 members, which can be divided into collagenases, gelatinases, stromelysins, matrilysins and the membrane-type MMPs. They have the ability to degrade the basement membrane of vessels, which is an essential requirement for tumor invasion into blood and lymphatic vessels [69]. MMP-1 expression has been found in a major population of proliferating Barrett’s and adenocarcinoma cells in vitro and may promote tumor growth [68]. It also correlates with lymph node metastasis and poor prognosis [68,69]. MMP-3, -7 and -9 have also been found to be prognostic biomarkers for EAC and MMP-9 is increased in the immature blood vessels seen in BE [69,70].

**ROS mediated mucosal damage**

ROS is constantly generated under normal conditions such as oxidative phosphorylation in the mitochondria or T-cell activation [78]. Increased levels of ROS are seen in esophagitis and BE and may be relevant to tumor initiation, growth and survival [79]. One mechanism by which this occurs is the induction of double-strand DNA breaks. In vitro, exposure of benign Barrett’s epithelial cell lines to acid results in ROS production and causes a time-dependent increase in levels of phospho-H2AX – a marker of double-strand DNA breaks [80]. Superoxide dismutase (SOD) and the glutathione reduct system are each considered to play a role in the defense mechanism against oxidative stress. Levels of glutathione and SOD observed in BE are lower than in normal esophageal mucosa [81]. Myeloperoxidase is released from the cytoplasmic granules of neutrophils and monocytes and amplifies the oxidative potential of hydrogen peroxide by generating highly reactive species such as hypochlorous acid [82]. Concentrations are higher in BE, and it is hypothesized to play a crucial role in carcinogenesis [83].

PDGF receptor and EGF receptors signal in part via ROS-dependent mechanisms [84,85]. Activation of these receptors results in the production of phosphatidylinositol 3,4,5-triphosphate and Akt activation, which plays a role in cellular proliferation and inhibition of apoptosis. Phosphatidylinositol 3,4,5-triphosphate also activates NADPH oxidase, which can be converted to H2O2 by SOD and result in a major source of O2·−.

Hypoxia is a common state in tumors, resulting in induction of hypoxia-inducible factors (HIF-1 and -2); resultant reoxygenation can result in significant oxidative stress via ROS, nitric oxide and H2O2 production [7]. Nitric oxide can increase invasiveness of dysplastic and cancerous cells via regulation of MMPs and tissue inhibitor of metalloproteinases [87]. HIF-1α is increased in BE and correlates with the degree of inflammation, but since this does not increase in dysplasia and neoplasia, it is hypothesized to be an early event in neoplastic progression and inflammation [87]. HIF-2α on the other hand is increased in dysplasia and further in EAC, while it is not expressed in BE, suggesting that it is active later in the neoplastic process [88]. Considered collectively, there is significant evidence to support the hypothesis of oxidative stress playing a role in the progression of esophageal inflammation to neoplasia.

**Inflammation induces genomic instability**

Widespread genomic instability is believed to facilitate neoplastic progression in BE. The process is possibly facilitated by the loss and mutation of cell cycle checkpoint machinery and tumor-suppressor
loci such as p16 and p53 [30]. There is however controversy regarding the timing of changes in tumor-suppressor genes in relation to genomic instability with some evidence to suggest that it occurs prior to changes in p53 and adenomatous polyposis coli [91]. Shortened telomere length and chromosomal instability have been demonstrated using fluorescence in situ hybridization in BE [20,93]. It has been demonstrated using quantitative FISH that carcinoma in situ of the esophagus arises from epithelium with short telomeres and chromosomal instability [94]. Additionally, copy number alteration and/or loss of heterozygosity at chromosomal fragile sites such as FRA3B are frequent and early events in BE supportive of the concept of a specific DNA damage profile attributable to Barrett’s [95]. Examination of sister chromatid exchange and micronucleus frequencies in BE demonstrates a significant increase of both in Barrett’s patients compared with controls, supporting the hypothesis of deficiency in DNA repair capacity [95].

NF-κB activation in inflamed epithelial cells is involved not only in the regulation of cell survival, proliferation and growth, but it also regulates activation-induced cytokine deaminase (AID), a nucleotide-editing enzyme which is directly involved in DNA instability [96]. Specific to the esophagus, NF-κB is activated by bile components and AID is aberrantly expressed in the columnar cell-lined BE in addition to Barrett’s adenocarcinoma, while low levels of expression are observed in normal squamous epithelial cells [53,37]. Furthermore, in vitro experiments using non-neoplastic esophageal squamous-derived cells showed that exposure to deoxycholic acid induces endogenous AID expression via NF-κB activation [96].

Profiling of the genomic landscape of EAC has revealed a total of 117 genes with predicted coding alterations, of which potentially actionable coding mutations were identified in 67 [98]. The most frequently mutated genes identified were TP53, SYNE1 and ARID1A. Although these mutations are hypothesized to be later events and necessary for tumor initiation, their identification does identify potential new therapeutic targets [98]. Studies examining the genomic differences between EAC and squamous cell carcinoma revealed substantial disparity in the spectrum of mutations between histological types and interestingly that the majority of mutations present in adenocarcinoma were already present in matched BE samples [99]. Analysis of data from high-density genomic profiling arrays identified focal RUNX1 deletions at 21q22.12 in 15% of adenocarcinomas examined. RUNX1 is known to behave as a tumor-suppressor gene in leukemia, thus making it an interesting focus. The potential function of RUNX1 was then further evaluated by reintroduction into an OE33 cell line carrying a deletion, resulting in a 69% reduction in anchorage-independent growth and supporting a potential role as a tumor suppressor in EAC [100].

Recombinase (hRAD51) is a key component of homologous recombination and repair in evolving genomic changes. Expression of RAD51 is elevated in Barrett’s adenocarcinoma cell lines and tissue specimens relative to normal cells, and its suppression was demonstrated to significantly prevent Barrett’s adenocarcinoma cells from acquiring genomic changes to either copy number or heterozygosity in several independent experiments employing single-nucleotide polymorphism arrays [101]. It is therefore likely that hRAD51 contributes significantly to genomic evolution during serial propagation of these cells and correlates with disease progression.

**Reciprocal mechanisms link energy metabolism with inflammation in the esophagus**

Alteration in energy metabolism pathways is one of the new emerging hallmarks of cancer and disease progression [102]. Various studies linking energy metabolism with angiogenesis, hypoxia and inflammation all highlight how dual processes, such as energy metabolism and inflammation, can act jointly to significantly alter the local microenvironment and attenuate disease progression [103–106]. While little is known about the state of energy metabolism in Barrett’s metaplasia, even less is known about how energy metabolism and inflammation cooperate to facilitate metaplastic progression, despite some current insight into metabolic signatures in esophageal cancer [107].

The proinflammatory cytokine IL-6, known to inhibit apoptosis, enhances glycolysis by activating STAT3, thus increasing the expression of two key glycolytic enzymes, hexokinase 2 and PFKFB3 [108]. In one study, secreted levels of IL-6 and STAT3 were found in Barrett’s tissue compared to normal adjacent squamous epithelium [55]. In addition to increased IL-6 mRNA in the Barrett’s tissue, immunological studies confirmed increased IL-6 expression in the intestinal glandular epithelium [59]. Therefore, increased expression of IL-6 may lead to activation of STAT3 and a subsequent increase in expression of anti-apoptotic genes and glycolytic pathway components. Tumor-suppressor protein p53 mediates the expression of a number of genes resulting in an increased rate of oxidative phosphorylation. It is well characterized as being mutated in Barrett’s tissue from metaplasia to dysplasia and adenocarcinoma [109,110]. Interestingly, unlike wild-type p53, mutated p53 enhances IL-6 promoter activity and thus may play a role in the upregulation of IL-6 in Barrett’s metaplasia [111]. In addition, p53 has been shown to indirectly regulate glycolysis through NF-κB [112]. NF-κB can organize energy metabolism networks by controlling the balance between oxidative and glycolytic pathways through the upregulation of mitochondrial synthesis of cytochrome c oxidase 2 [112]. p53 also links inflammation with energy metabolism in an HIF1α-dependent interaction with TP53-inducible glycolysis and apoptosis regulator, a key mediator of glycolysis under hypoxic conditions [109]. Moreover, HIF1α protein expression is associated with the inflammatory processes in Barrett’s metaplasia [88]. Therefore, further studies linking inflammation with energy metabolism through some of these pathways in the esophagus would enhance our understanding of the mechanisms involved in tumor progression and allow more accurate diagnosis of pre-neoplastic lesions more amenable to cancer development.

**EAC demonstrates a unique miRNA signature**

miRNAs are small non-coding RNAs that typically inhibit the translation and stability of messenger mRNAs controlling
genes involved in cellular processes such as inflammation, cell-cycle regulation, stress response, differentiation, apoptosis and migration [114]. They have been found to play an essential role in the progression and development of cancer via their ability to function as oncogenes or tumor suppressors [115]. Inflammatory gene and miRNA signatures derived from tumor and adjacent non-tumor tissues have been demonstrated as a potentially useful prognostic classifier in Barrett’s-associated adenocarcinoma when used in combination [116]. Measuring the expression of 23 inflammation-associated genes in tumors and adjacent normal tissues from 93 patients using quantitative reverse transcription polymerase chain reaction, an inflammatory risk model has been reported with IFN-γ, IL-1α, IL-8, IL-21, IL-23 and proteoglycan expression in both tumor and non-tumor samples associated with poor prognosis. This relationship allowed generation of an inflammatory risk score, and when used in combination with miRNA-375 and the miR signature, an improved prognostic classifier was generated [117].

Histological subtype analysis of Barrett’s metaplasia has revealed metaplasia-specific signatures and identified five miRNAs, which are significantly dysregulated across the histological subtypes – two overexpressed has –miR-192, –miR-215 and three underexpressed has –miR-18a*, –miR-203 and –miR-205. Furthermore, the expression of three miRNAs (miR-99b and miR-199a-3p and -5p) has been demonstrated to be associated with the presence of lymph-node metastases, suggesting that miRNA profiling may provide a useful prognostic tool in the staging of esophageal cancer [117].

**Expert commentary**

Esophageal cancer continues to present a significant challenge to both translational research and clinical management. An unprecedented rise in obesity levels in the western world and concurrent rise in obesity-associated malignancy has forced investigators to scrutinize this important association. The rising incidence of adenocarcinoma of the esophagus highlights the importance of understanding the molecular mechanisms underpinning its association with inflammation to identify key chemopreventive strategies. A wide range of alterations in the tissue microenvironment, metabolic profile, intracellular signaling pathways and miRNA signatures contribute to Barrett’s-associated adenocarcinoma. Pharmacological strategies to reduce systemic inflammation investigated to date include aspirin and statins. Whilst there is currently insufficient evidence to recommend routine Aspirin use as a chemopreventive agent, the results from a number of key clinical trials such as the AspECT trial are hotly anticipated. This area of investigation will undoubtedly expand in the coming years and remains a key focus of both clinical and translational research.

**Five year view: current & future anti-inflammatory therapeutic targets**

**Pharmacological inhibition of COX-2**

As a NSAID compound, aspirin irreversibly inhibits both COX-1 and COX-2 isoenzymes. It has been demonstrated to produce a protective effect against EAC by a number of studies and a recently published meta-analysis of nine studies has confirmed this association and also suggested an increased degree of protection relative to the duration of usage [118]. As COX-2 induces the production of Th2 cytokines and reduces the Th1 cytokine levels, it could modulate the inflammatory disease sequence [119]. The AspECT trial was designed to examine the chemopreventive properties of both aspirin and acid-suppression therapy and is due to be completed in 2019 [118,120]. Until it is completed, there is currently insufficient evidence to recommend NSAIDs for use in the prevention of esophageal cancer.

**Anti-reflux therapy**

There is no evidence that either pharmacological or surgical anti-reflux therapy has the capacity to eradicate BE [121]. However, a multicenter prospective cohort of 540 patients with Barrett’s recently demonstrated that the risk of neoplastic progression was reduced during the follow-up period of 5.2 years [122]. Additional research has demonstrated the antioxidant and immune-modulatory properties of proton pump inhibitors [123]. Nonetheless, valid data supportive of any overall cancer preventative effect are limited [124].

**Weight loss**

Despite the epidemiological evidence linking obesity with cancer of the esophagus, the potentially therapeutic or preventative role of weight loss in the context is unknown. A systematic review of the incidence of esophageal cancer after bariatric surgery has revealed a deficit in the reported incidence in the setting of bariatric surgery [125]. Further studies are required to quantify the potential benefit of weight loss in reducing the risk of EAC.

**Statins**

There is evidence to suggest that statin, a hydroxymethylglutaryl-CoA reductase inhibitor (HMG-CoA), use is associated with a reduced risk of neoplastic progression in BE [126]. In vitro studies have demonstrated the ability of statins to inhibit proliferation and induce apoptosis in both malignant and non-malignant Barrett’s cell lines (OE33, Fao-1, NIHhERT) [127]. Their efficacy is thought to be due to a number of effects including reduction of Rac activity and inhibition of both extracellular signal-regulated kinase and protein kinase B (AKT). Additionally, statin treatment increased miRNA and protein expression of the anti-apoptotic proteins Bcl-2 and Bcl-xL [122]. A recent study has advocated the use of statins in combination with COX inhibition to demonstrate a reduced incidence of progression of BE to EAC [128]. A cost–benefit analysis concluded that in combination with aspirin, statins were an expensive form of chemoprevention but could be cost–effective in a cohort of patients at higher risk of progression to EAC [129]. Randomized controlled trials are required to further determine whether statins have chemopreventive effects in high-risk groups [130].

**Dietary chemoprevention**

Examination of the modulatory effect of Omega-3 fatty acids on BE is currently underway and a Phase IV double-blind randomized controlled trial is due for completion in 2014 [131]. This is based on the ability of both eicosapentenoic acid and
docosahexaenoic acid to compete with arachidonic acid for the COX enzyme, thus inhibiting its metabolism [132]. Eicosapentaenoic acid has been shown to be associated with preservation of lean body mass post esophagectionomy, demonstrating its ability to modulate immune function and limit catabolism in advanced cancer [133].

Honokiol, a phenol in herbal tea, has pro-apoptotic effects associated with the inhibition of STAT3. It has been shown to increase necrosis and apoptosis in transformed but not in non-transformed Barrett's cells and exhibit a similar effect on adenocarcinoma cells [134]. There is a great deal of preliminary work required on the effect of STAT3 inhibition in BE and EAC; however, it remains a potential future therapeutic target.

Curcumin, the yellow pigment derived from turmeric, has been a recent focus of investigation. It has been suggested to possess a number of health benefits such as anti-inflammatory and anti-carcinogenic properties [135]. Significant abrogation of DNA damage and NF-κB activation produced by bile exposure has been demonstrated in vitro with curcumin pre-treatment capable of abolishing the ability of deoxycholic acid to activate NF-κB [136]. The cytotoxic properties of curcumin are associated with accumulation in the G2/M cell-cycle phases and chromatin morphology consistent with mitotic catastrophe, indicating a non-apoptotic mechanism of action for the compound. It also doubles apoptotic frequency in vivo in Barrett's epithelial cells [79,135].

**Antioxidant therapy**

Administration of a number of antioxidants has been shown to prevent mucosal damage in models of esophagitis, suggesting a role for anti-oxidant therapy in that setting [136]. *In vitro* studies have demonstrated that treatment with antioxidants such as vitamin C and C-PTIO (2-[4-Carbomethoxyphenyl]-4,4,5,5-tetramethylimidazole-1-oxyl-3-oxide) can prevent DNA damage by bile acid in OE33 cells and indeed are potentially more effective used in combination with acid-suppression therapy [137]. Further epidemiological studies have demonstrated a potential link between the intake of β-carotene and decreased risk of dysplastic BE [138]. While it remains an interesting potential treatment strategy, further studies are required to investigate the potential of antioxidant therapy.

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Papers of special note have been highlighted as:

* of interest
** of considerable interest

3. Conclusive evidence of the rising trend in the incidence of oesophageal adenocarcinoma.


- Pooled results of nine studies (two cohorts, seven case control) containing 1813 cancer cases were identified. Statistical pooling showed a protective association between any use of aspirin/NSAID and oesophageal cancer (odds ratio [OR] 0.57, 95% CI 0.47-0.71). Both intermittent (OR 0.82; CI 0.67-0.99) and frequent medication use were protective (OR 0.54; CI 0.43-0.67), with greater protection with more frequent use. Any use was protective against both oesophageal adenocarcinoma (OR 0.67; CI 0.51-0.87).


- The AspECT trial is the biggest, multicentre, randomized controlled clinical trial looking at the long-term chemoprevention effect of esomprazole with or without aspirin. More than 85% of the participants tolerated the medications at the initial intended dose, and the drop-out rate has been 7%. Results of this trial will be of great interest.


- Immunohistochemical analysis showed that STAT3 is expressed in dysplastic Barrett’s esophagus and cancer tissue. Treatment of Seg.1 cells with media containing bile acid cocktail and acidified to pH 4 resulted in increased activation of STAT3 and IL-6 secretion and increased expression of Bcl-x(L). Inhibition of the STAT3 pathway resulted in increased apoptosis.


- Key study reviewing the epidemiological association between obesity and cancer. It is suggested that successful intervention strategies for weight loss and maintenance are needed for reduction in cancer risk.


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