The role of inflammation in cancer of the esophagus

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Abstract

Esophageal adenocarcinoma is the eighth most common malignancy worldwide. The overall prognosis is poor, with 5-year survival ranges 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] [3]. Epidemiologically, the rise parallels the increasing prevalence of gastroesophageal reflux disease (GERD), Barrett's esophagus (BE) and obesity in affected societies [3,4]. EAC is currently the sixth leading cause of cancer death worldwide [5]. The overall prognosis is poor, with l 5-year survival ranging from 15 to 25% and between 30 and 50% in patients that can be treated with curative intent [6].

EAC has been described as an 'exemplar model' of inflammation-associated cancer [7]. 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 [8]. 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 [9,10]. 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 [11]. Virchow in 1863 observed the presence of leucocytes in malignant tissue and first linked cancer with a state of chronic inflammation [12]. Increased understanding of the inflammatory microenvironment has produced evidence supportive of the role of inflammation in tumorigenesis. As

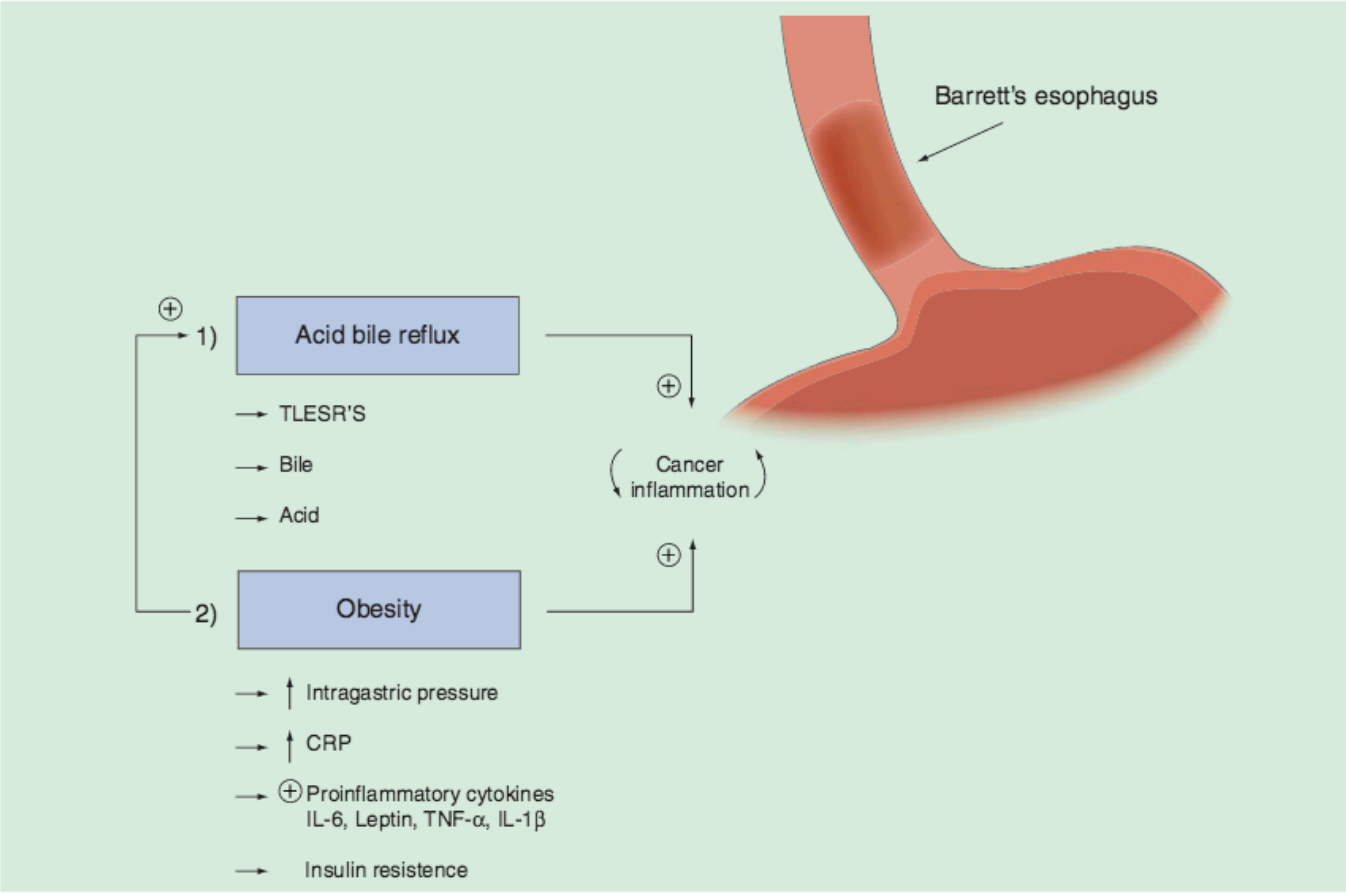

Figure 1. Factors contributing to inflammation of the lower oesophagus. Acid/bile reflux results from transient relaxations at the lower esophageal sphincter therefore exposing the oesophageal mucosa to bile and acid.
CRP: C-reactive protein; TLESRs: Transient lower esophageal sphincter relaxations.

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 [14]. 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 esophagojejunal 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 [34]. 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 tumorigenic 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 IKKβ. 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-8 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

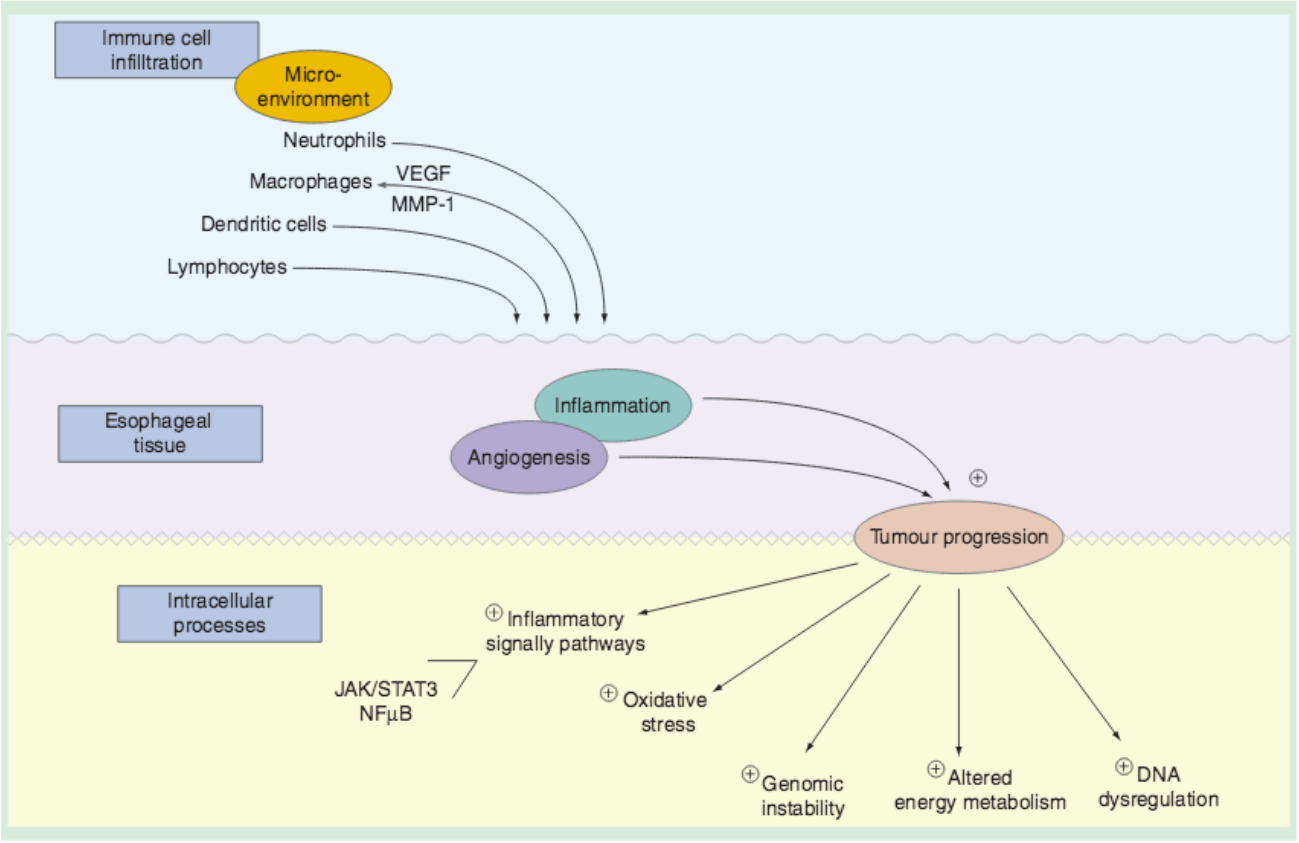

Figure 2. Environmental, local and intracellular processes resulting from inflammation in the oesophagus which facilitate tumour initiation and progression.
MMP: Matrix metalloproteinase.

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) [10,60]. 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 vitro*, myeloid dendritic cells co-cultured with BE and EAC cells stimulate regulatory T cell-differentiation from naïve $CD4^+$ T cells promoting tumor progression [63]. Metaplastic 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 metalloproteinase (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]. Thrombospondin-1 is another matricellular protein with angiogenic effects and the capacity to regulate TGF-β_1. 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 [68]. 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].

Transcription factors mediate tumor promotion & metastasis

The major inflammatory signaling pathways associated with cancer progression in the esophagus are NF-κB and STAT 3. STAT3 mediates the activity of a number of cytokines involved with cancer-promoting inflammatory responses by promoting at least three hallmarks of cancer, namely proliferation, survival and angiogenesis [71]. Closely integrated with NF-κB signaling, STAT3 activation occurs secondary to binding of a wide range of cytokines including IL-2, IL-6, IL-9, IL-10, IL-11, IL-15, IL-17 and leptin to their cognate receptor with subsequent phosphorylation, dimerization and nuclear translocation. Binding to specific DNA sites, gene transcription is induced within 30–60 min of activation producing up-regulation of a number of proinflammatory, pro-metastatic, pro-angiogenic and anti-apoptotic genes, as well as initiation of a positive feed-forward loop resulting in further STAT3 expression [72,73].

STAT3 may also link obesity with esophageal inflammation, as a number of the proinflammatory cytokines activating STAT3 signaling are secreted by adipose tissue including leptin, IL-6, IL-8, IL-1β, oncostatin M, TNF-α and MCP-1, [71],. In addition, the role of bile and gastric acids in STAT3/NF-κB signaling has been studied in HET-1A cells indicating increased activation of both following exposure compared with parental cells and supporting a role for bile and acid reflux at a signaling level [74]. Activated phospho-STAT3 has been demonstrated only in transformed Barrett's cells with resultant inhibition of apoptosis [75]. STAT3 knockdown has been demonstrated to reduce cell proliferation and migration in Barrett's adenocarcinoma [76].

The NF-κB family consists of five Rel proteins and persistent activation of one, RELA, is STAT3 dependent. RELA codes for a number of cytokines and growth factors that in turn activate STAT3 resulting in a positive feed-forward loop [71]. NF-κB transcription is up-regulated along the sequence of BE to EAC with concurrent down-regulation of its negative regulator I-κB [77].

ROS mediate 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 redox 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 H_2O_2 by SOD and result in a major source of O_2^- [86].

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 H_2O_2 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 [88]. 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 [89]. 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 p16 and p53 [90]. 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 [92,93]. 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 [90]. 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 cytidine 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 [53,97]. 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 (hsRAD51) 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 hsRAD51 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 [55]. 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-κβ [112]. NF-κβ 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 [113]. 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 [116].

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 –miR18a*, –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 chemopreventative 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 chemopreventative 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 chemopreventative properties of both aspirin and acid-suppression therapy and is due to be completed in 2019 [8,120]. 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, Flo-1, QhERT) [127]. Their efficacy is thought to be due to a number of effects including reduction of Ras activity and inhibition of both extracellular signal-regulated kinase and protein kinase B (AKT). Additionally, statin treatment increased mRNA and protein expression of the anti-apoptotic proteins Bax and Bad [127]. 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 chemopreventative 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]. Eicosapentenoic acid has been shown to be associated with preservation of lean body mass post esophagectomy, demonstrating its ability to modulate immune function and limit catabolism in advanced cancer [133].

Honokiol, a polyphenol 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-oxidant, 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 [135]. 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-Carboxylphenyl]-4,4,5,5,-tetramethylimidazoline-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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References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1. Vizcaino AP, Moreno V, Lambert R, Parkin DM. Time trends incidence of both major histologic types of esophageal carcinomas in selected countries, 1973-1995. Int J Cancer 2002;99(6):860-8
2. Dubecz A, Solymosi N, Stadlhuber RJ, et al. Does the incidence of adenocarcinoma of the esophagus and gastric cardia continue to rise in the twenty-first Century?-a SEER Database Analysis. J Gastrointest Surg 2013. [Epub ahead of print]

• **Conclusive evidence of the rising trend in the incidence of oesophageal adenocarcinoma.**

3. Brown LM, Devesa SS, Chow WH. Incidence of adenocarcinoma of the esophagus among white Americans by sex, stage, and age. J Natl Cancer Inst 2008; 100(16):1184-7
4. Edelstein ZR, Farrow DC, Bronner MP, et al. Central adiposity and risk of Barrett's esophagus. Gastroenterology 2007;133(2): 403-11
5. O'Neill JR, Stephens NA, Save V, et al. Defining a positive circumferential resection margin in oesophageal cancer and its implications for adjuvant treatment. Br J Surg 2013;100(8):1055-63

6. Enzinger PC, Mayer RJ. Esophageal cancer. N Engl J Med 2003;349(23):2241-52
7. Picardo SL, Maher SG, O'Sullivan JN, Reynolds JV. Barrett's to oesophageal cancer sequence: a model of inflammatory-driven upper gastrointestinal cancer. Dig Surg 2012;29(3):251-60
8. Nicholson A, Jankowski J. Acid reflux and oesophageal cancer. Recent Results Cancer Res 2011;185:65-82
9. Corley DA, Kerlikowske K, Verma R, Buffler P. Protective association of aspirin/NSAIDs and esophageal cancer: a systematic review and meta-analysis. Gastroenterology 2003;124(1):47-56

- **Pooled results of nine studies (two cohort, seven case control) containing 1813 cancer cases were identified. Statistical pooling showed a protective association between any use of aspirin/NSAID and esophageal 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 esophageal adenocarcinoma (OR: 0.67; CI: 0.51–0.87).**

10. Das D, Chilton AP, Jankowski JA. Chemoprevention of oesophageal cancer and the AspECT trial. Recent Results Cancer Res 2009;181:161-9

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

11. Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med 1986;315(26):1650-9
12. Keibel A, Singh V, Sharma MC. Inflammation, microenvironment, and the immune system in cancer progression. Curr Pharm Des 2009;15(17):1949-55
13. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet 2001;357(9255):539-45
14. Negus RP, Stamp GW, Hadley J, Balkwill FR. Quantitative assessment of the leukocyte infiltrate in ovarian cancer and its relationship to the expression of C-C chemokines. Am J Pathol 1997;150(5):1723-34
15. Brigati C, Noonan DM, Albini A, Benelli R. Tumors and inflammatory infiltrates: friends or foes? Clin Exp Metastasis 2002;19(3):247-58
16. Burke F, Relf M, Negus R, Balkwill F. A cytokine profile of normal and malignant ovary. Cytokine 1996;8(7):578-85
17. Poehlmann A, Kuester D, Malfertheiner P, et al. Inflammation and Barrett's carcinogenesis. Pathol Res Pract 2012;208(5):269-80
18. Abdel-Latif MM, Duggan S, Reynolds JV, Kelleher D. Inflammation and esophageal carcinogenesis. Curr Opin Pharmacol 2009;9(4):396-404
19. Newton M, Bryan R, Burnham WR, Kamm MA. Evaluation of Helicobacter pylori in reflux oesophagitis and Barrett's oesophagus. Gut 1997;40(1):9-13
20. Freedman J, Lindqvist M, Hellstrom PM, et al. Presence of bile in the oesophagus is associated with less effective oesophageal motility. Digestion 2002;66(1):42-8
21. Hopwood D, Bateson MC, Milne G, Bouchier IA. Effects of bile acids and hydrogen ion on the fine structure of oesophageal epithelium. Gut 1981;22(4):306-11
22. Nehra D, Howell P, Williams CP, et al. Toxic bile acids in gastro-oesophageal reflux disease: influence of gastric acidity. Gut 1999;44(5):598-602
23. Nishijima K, Miwa K, Miyashita T, et al. Impact of the biliary diversion procedure on carcinogenesis in Barrett's esophagus surgically induced by duodenoesophageal reflux in rats. Ann Surg 2004;240(1):57-67
24. Dvorak K, Chavarria M, Payne CM, et al. Activation of the interleukin-6/STAT3 antiapoptotic pathway in esophageal cells by bile acids and low pH: relevance to Barrett's esophagus. Clin Cancer Res 2007;13(18 Pt 1):5305-13

- **Immunohistochemical analysis showed that STAT3 is expressed in dysplastic Barrett's esophagus and cancer tissues. 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.**

25. Fitzgerald RC, Omary MB, Triadafilopoulos G. Dynamic effects of acid on Barrett's esophagus. An ex vivo proliferation and differentiation model. J Clin Invest 1996;98(9):2120-8
26. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420(6917):860-7
27. Garcia JM, Splenser AE, Kramer J, et al. Circulating inflammatory cytokines and adipokines are associated with increased risk of Barrett's esophagus: a case-control study. Clin Gastroenterol Hepatol 2014;12(2):229-38.e3
28. Jenkins GJ, Harries K, Doak SH, et al. The bile acid deoxycholic acid [DCA] at neutral pH activates NF-kappaB and induces IL-8 expression in oesophageal cells in vitro. Carcinogenesis 2004;25(3):317-23
29. Deans DA, Wigmore SJ, Gilmour H, et al. Elevated tumour interleukin-1beta is associated with systemic inflammation: a marker of reduced survival in gastro-oesophageal cancer. Br J Cancer 2006;95(11):1568-75
30. Singh S, Sharma AN, Murad MH, et al. Central adiposity is associated with increased risk of esophageal inflammation, metaplasia, and adenocarcinoma: a systematic review and meta-analysis. Clin Gastroenterol Hepatol 2013;11(11):1399-412; e7
31. El-Serag HB, Graham DY, Satia JA, Rabeneck L. Obesity is an independent risk factor for GERD symptoms and erosive esophagitis. Am J Gastroenterol 2005;100(6):1243-50
32. Anggiansah R, Sweis R, Anggiansah A, et al. The effects of obesity on oesophageal function, acid exposure and the symptoms of gastro-oesophageal reflux disease. Aliment Pharmacol Ther 2013;37(5):555-63
33. Galic S, Oakhill JS, Steinberg GR. Adipose tissue as an endocrine organ. Mol Cell Endocrinol 2010;316(2):129-39
34. Das UN. Is obesity an inflammatory condition? Nutrition 2001;17(11-12):953-66
35. Monteiro R, Azevedo I. Chronic inflammation in obesity and the metabolic syndrome. Mediators Inflamm 2010;2010
36. Snijder MB, van Dam RM, Visser M, Seidell JC. What aspects of body fat are particularly hazardous and how do we measure them? Int J Epidemiol 2006;35(1):83-92
37. Wajchenberg BL, Giannella-Neto D, da Silva ME, Santos RF. Depot-specific hormonal characteristics of subcutaneous and visceral adipose tissue and their relation to the metabolic syndrome. Horm Metab Res 2002;34(11-12):616-21

38. Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer 2004;4(8):579-91

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

39. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 1988;37(12):1595-607

40. Moller DE, Flier JS. Insulin resistance–mechanisms, syndromes, and implications. N Engl J Med 1991;325(13):938-48

41. Donohoe CL, Doyle SL, McGarrigle S, et al. Role of the insulin-like growth factor 1 axis and visceral adiposity in oesophageal adenocarcinoma. Br J Surg 2012;99(3): 387-96

42. Doyle SL, Donohoe CL, Finn SP, et al. IGF-1 and its receptor in esophageal cancer: association with adenocarcinoma and visceral obesity. Am J Gastroenterol 2012; 107(2):196-204

43. Doyle SL, Donohoe CL, Lysaght J, Reynolds JV. Visceral obesity, metabolic syndrome, insulin resistance and cancer. Proc Nutr Soc 2012;71(1):181-9

44. Aguirre V, Uchida T, Yenush L, et al. The c-Jun NH[2]-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser[307]. J Biol Chem 2000;275(12):9047-54

45. Cai D, Yuan M, Frantz DF, et al. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. Nat Med 2005;11(2):183-90

46. Keaney JF Jr, Larson MG, Vasan RS, et al. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. Arterioscler Thromb Vasc Biol 2003;23(3):434-9

47. Duggan C, Onstad L, Hardikar S, et al. Association between markers of obesity and progression from Barrett's esophagus to esophageal adenocarcinoma. Clin Gastroenterol Hepatol 2013;11(8):934-43

48. Howard JM, Beddy P, Ennis D, et al. Associations between leptin and adiponectin receptor upregulation, visceral obesity and tumour stage in oesophageal and junctional adenocarcinoma. Br J Surg 2010;97(7): 1020-7

49. Howard JM, Pidgeon GP, Reynolds JV. Leptin and gastro-intestinal malignancies. Obes Rev 2010;11(12):863-74

50. Ryan AM, Healy LA, Power DG, et al. Barrett esophagus: prevalence of central adiposity, metabolic syndrome, and a proinflammatory state. Ann Surg 2008; 247(6):909-15

51. Fitzgerald RC, Abdalla S, Onwuegbusi BA, et al. Inflammatory gradient in Barrett's oesophagus: implications for disease complications. Gut 2002;51(3):316-22

52. Quante M, Bhagat G, Abrams JA, et al. Bile acid and inflammation activate gastric cardia stem cells in a mouse model of Barrett-like metaplasia. Cancer Cell 2012; 21(1):36-51

53. O'Riordan JM, Abdel-latif MM, Ravi N, et al. Proinflammatory cytokine and nuclear factor kappa-B expression along the inflammation-metaplasia-dysplasia-adenocarcinoma sequence in the esophagus. Am J Gastroenterol 2005;100(6):1257-64

54. Tselepis C, Perry I, Dawson C, et al. Tumour necrosis factor-alpha in Barrett's oesophagus: a potential novel mechanism of action. Oncogene 2002;21(39):6071-81

55. Dvorakova K, Payne CM, Ramsey L, et al. Increased expression and secretion of interleukin-6 in patients with Barrett's esophagus. Clin Cancer Res 2004;10(6): 2020-8

56. Hong S, Lee HJ, Kim SJ, Hahm KB. Connection between inflammation and carcinogenesis in gastrointestinal tract: focus on TGF-beta signaling. World J Gastroenterol 2010;16(17):2080-93

57. von Rahden BH, Stein HJ, Feith M, et al. Overexpression of TGF-beta1 in esophageal [Barrett's] adenocarcinoma is associated with advanced stage of disease and poor prognosis. Mol Carcinog 2006;45(10): 786-94

58. Mu Y, Gudey SK, Landstrom M. Non-Smad signaling pathways. Cell Tissue Res 2012;347(1):11-20

59. Onwuegbusi BA, Aitchison A, Chin SF, et al. Impaired transforming growth factor beta signalling in Barrett's carcinogenesis due to frequent SMAD4 inactivation. Gut 2006;55(6):764-74

60. Buskens CJ, Ristimaki A, Offerhaus GJ, et al. Role of cyclooxygenase-2 in the development and treatment of oesophageal adenocarcinoma. Scand J Gastroenterol Suppl 2003(239):87-93

61. Shirvani VN, Ouatu-Lascar R, Kaur BS, et al. Cyclooxygenase 2 expression in Barrett's esophagus and adenocarcinoma: ex vivo induction by bile salts and acid exposure. Gastroenterology 2000;118(3): 487-96

62. Mobius C, Stein HJ, Spiess C, et al. COX2 expression, angiogenesis, proliferation and survival in Barrett's cancer. Eur J Surg Oncol 2005;31(7):755-9

63. Somja J, Demoulin S, Roncarati P, et al. Dendritic cells in Barrett's esophagus carcinogenesis: an inadequate microenvironment for antitumor immunity? Am J Pathol 2013;182(6):2168-79

64. Harrison RF, Perry I, Jankowski JA. Barrett's mucosa: remodelling by the microenvironment. J Pathol 2000;192(1): 1-3

65. Salmela MT, Karjalainen-Lindsberg ML, Puolakkainen P, Saarialho-Kere U. Upregulation and differential expression of matrilysin [MMP-7] and metalloelastase [MMP-12] and their inhibitors TIMP-1 and TIMP-3 in Barrett's oesophageal adenocarcinoma. Br J Cancer 2001;85(3):383-92

66. Fitzgerald RC, Onwuegbusi BA, Bajaj-Elliott M, et al. Diversity in the oesophageal phenotypic response to gastro-oesophageal reflux: immunological determinants. Gut 2002;50(4):451-9

67. Sage H, Decker J, Funk S, Chow M. SPARC: a Ca2+-binding extracellular protein associated with endothelial cell injury and proliferation. J Mol Cell Cardiol 1989;21(Suppl 1)):13-22

68. Grimm M, Lazariotou M, Kircher S, et al. MMP-1 is a [pre-]invasive factor in Barrett-associated esophageal adenocarcinomas and is associated with positive lymph node status. J Transl Med 2010;8:99

69. Murray GI, Duncan ME, O'Neil P, et al. Matrix metalloproteinase-1 is associated with poor prognosis in oesophageal cancer. J Pathol 1998;185(3):256-61

70. Auvinen MI, Sihvo EI, Ruohtula T, et al. Incipient angiogenesis in Barrett's epithelium and lymphangiogenesis in Barrett's adenocarcinoma. J Clin Oncol 2002;20(13):2971-9

71. O'Sullivan KE, Reynolds JV, O'Hanlon C, et al. Could signal transducer and activator of transcription 3 be a therapeutic target in obesity-related gastrointestinal malignancy? J Gastrointest Cancer 2014;45(1):1-11

72. Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. Nat Rev Cancer 2009;9(11): 798-809

73. Jarnicki A, Putoczki T, Ernst M. Stat3: linking inflammation to epithelial cancer - more than a "gut" feeling? Cell Div 2010;5:14

74. Gibson MK, Dhaliwal AS, Clemons NJ, et al. Barrett's esophagus: cancer and molecular biology. Ann N Y Acad Sci 2013;1300:296-314
75. Zhang HY, Zhang Q, Zhang X, et al. Cancer-related inflammation and Barrett's carcinogenesis: interleukin-6 and STAT3 mediate apoptotic resistance in transformed Barrett's cells. Am J Physiol Gastrointest Liver Physiol 2011;300(3): G454-60
76. Timme S, Ihde S, Fichter CD, et al. STAT3 expression, activity and functional consequences of STAT3 inhibition in esophageal squamous cell carcinomas and Barrett's adenocarcinomas. Oncogene 2013. [Epub ahead of print]
77. Jenkins GJ, Mikhail J, Alhamdani A, et al. Immunohistochemical study of nuclear factor-kappaB activity and interleukin-8 abundance in oesophageal adenocarcinoma; a useful strategy for monitoring these biomarkers. J Clin Pathol 2007;60(11): 1232-7
78. Cadenas E, Davies KJ. Mitochondrial free radical generation, oxidative stress, and aging. Free Radic Biol Med 2000;29(3-4): 222-30
79. Kavanagh ME, O'Sullivan KE, O'Hanlon C, et al. The esophagitis to adenocarcinoma sequence; the role of inflammation. Cancer Lett 2014;345(2):182-9
80. Zhang HY, Hormi-Carver K, Zhang X, et al. In benign Barrett's epithelial cells, acid exposure generates reactive oxygen species that cause DNA double-strand breaks. Cancer Res 2009;69(23):9083-9
81. Peters WH, Roelofs HM, Hectors MP, et al. Glutathione and glutathione S-transferases in Barrett's epithelium. Br J Cancer 1993;67(6):1413-17
82. Klebanoff SJ. Myeloperoxidase. Proc Assoc Am Physicians 1999;111(5):383-9
83. Sihvo EI, Salminen JT, Rantanen TK, et al. Oxidative stress has a role in malignant transformation in Barrett's oesophagus. Int J Cancer 2002;102(6):551-5
84. Sundaresan M, Yu ZX, Ferrans VJ, et al. H2O2 for platelet-derived growth factor signal transduction. Science 1995; 270(5234):296-9
85. Bae YS, Kang SW, Seo MS, et al. Epidermal growth factor [EGF]-induced generation of hydrogen peroxide. Role in EGF receptor-mediated tyrosine phosphorylation. J Biol Chem 1997;272(1):217-21
86. Fruehauf JP, Meyskens FL Jr. Reactive oxygen species: a breath of life or death? Clin Cancer Res 2007;13(3):789-94
87. Clemons NJ, Shannon NB, Abeyratne LR, et al. Nitric oxide-mediated invasion in Barrett's high-grade dysplasia and adenocarcinoma. Carcinogenesis 2010;31(9): 1669-75
88. Ling FC, Khochfar J, Baldus SE, et al. HIF-1alpha protein expression is associated with the environmental inflammatory reaction in Barrett's metaplasia. Diseases of the esophagus : official journal of the International Society for Diseases of the Esophagus/ ISDE 2009;22(8):694-9
89. Griffiths EA, Pritchard SA, McGrath SM, et al. Increasing expression of hypoxia-inducible proteins in the Barrett's metaplasia-dysplasia-adenocarcinoma sequence. Br J Cancer 2007;96(9): 1377-83
90. Lai LA, Kostadinov R, Barrett MT, et al. Deletion at fragile sites is a common and early event in Barrett's esophagus. Mol Cancer Res 2010;8(8):1084-94
91. Yang Y, Fruehauf J, Xiang S, Li CJ. Genomic instability in precancerous lesions before inactivation of tumor suppressors p53 and APC in patients. Cell Cycle 2006; 5(13):1443-7
92. O'Sullivan JN, Finley JC, Risques RA, et al. Telomere length assessment in tissue sections by quantitative FISH: image analysis algorithms. Cytometry A 2004; 58(2):120-31
93. Finley JC, Reid BJ, Odze RD, et al. Chromosomal instability in Barrett's esophagus is related to telomere shortening. Cancer Epidemiol Biomarkers Prev 2006; 15(8):1451-7
94. Takubo K, Fujita M, Izumiyama N, et al. Q-FISH analysis of telomere and chromosome instability in the oesophagus with and without squamous cell carcinoma in situ. J Pathol 2010;221(2):201-9
95. Karaman A, Binici DN, Kabalar ME, et al. Genomic instability in patients with Barrett's esophagus. Cancer Genet Cytogenet 2010;201(2):88-93
96. Shimizu T, Marusawa H, Endo Y, Chiba T. Inflammation-mediated genomic instability: roles of activation-induced cytidine deaminase in carcinogenesis. Cancer Sci 2012;103(7):1201-6
97. Morita S, Matsumoto Y, Okuyama S, et al. Bile acid-induced expression of activation-induced cytidine deaminase during the development of Barrett's oesophageal adenocarcinoma. Carcinogenesis 2011;32(11):1706-12
98. Chong IY, Cunningham D, Barber LJ, et al. The genomic landscape of oesophagogastric junctional adenocarcinoma J Pathol 2013;231(3):301-10
99. Agrawal N, Jiao Y, Bettegowda C, et al. Comparative genomic analysis of esophageal adenocarcinoma and squamous cell carcinoma. Cancer Discov 2012;2(10): 899-905
100. Dulak AM, Schumacher SE, van Lieshout J, et al. Gastrointestinal adenocarcinomas of the esophagus, stomach, and colon exhibit distinct patterns of genome instability and oncogenesis. Cancer Res 2012;72(17): 4383-93
101. Pal J, Bertheau R, Buon L, et al. Genomic evolution in Barrett's adenocarcinoma cells: critical roles of elevated hsRAD51, homologous recombination and Alu sequences in the genome. Oncogene 2011; 30(33):3585-98
102. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144(5):646-74
103. De Bock K, Georgiadou M, Carmeliet P. Role of endothelial cell metabolism in vessel sprouting. Cell Metab 2013;18(5):634-47
104. Shi LZ, Wang R, Huang G, et al. HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. J Exp Med 2011;208(7):1367-76
105. Straus DS. TNFalpha and IL-17 cooperatively stimulate glucose metabolism and growth factor production in human colorectal cancer cells. Mol Cancer 2013;12:78
106. Tornatore L, Thotakura AK, Bennett J, et al. The nuclear factor kappa B signaling pathway: integrating metabolism with inflammation. Trends Cell Biol 2012; 22(11):557-66
107. Zhang X, Xu L, Shen J, et al. Metabolic signatures of esophageal cancer: NMR-based metabolomics and UHPLC-based focused metabolomics of blood serum. Biochim Biophys Acta 2013;1832(8):1207-16
108. Ando M, Uehara I, Kogure K, et al. Interleukin 6 enhances glycolysis through expression of the glycolytic enzymes hexokinase 2 and 6-phosphofructo-2-kinase/ fructose-2,6-bisphosphatase-3. J Nippon Med Sch 2010;77(2):97-105
109. Cheung EC, Ludwig RL, Vousden KH. Mitochondrial localization of TIGAR under hypoxia stimulates HK2 and lowers ROS and cell death. Proc Natl Acad Sci USA 2012. 109(50):20491-6
110. Kastelein F, Biermann K, Steyerberg EW, et al. Aberrant p53 protein expression is associated with an increased risk of

neoplastic progression in patients with Barrett's oesophagus. Gut 2013;62(12): 1676-83

111. Angelo LS, Talpaz M, Kurzrock R. Autocrine interleukin-6 production in renal cell carcinoma: evidence for the involvement of p53. Cancer Res 2002;62(3):932-40
112. Kawauchi K, Araki K, Tobiume K, Tanaka N. p53 regulates glucose metabolism through an IKK-NF-kappaB pathway and inhibits cell transformation. Nat Cell Biol 2008;10(5):611-18
113. Mauro C, Leow SC, Anso E, et al. NF-kappaB controls energy homeostasis and metabolic adaptation by upregulating mitochondrial respiration. Nat Cell Biol 2011;13(10):1272-9
114. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006;6(11):857-66
115. Zhang B, Pan X, Cobb GP, Anderson TA. microRNAs as oncogenes and tumor suppressors. Dev Biol 2007;302(1):1-12
116. Nguyen GH, Schetter AJ, Chou DB, et al. Inflammatory and microRNA gene expression as prognostic classifier of Barrett's-associated esophageal adenocarcinoma. Clin Cancer Res 2010; 16(23):5824-34
117. Feber A, Xi L, Pennathur A, et al. MicroRNA prognostic signature for nodal metastases and survival in esophageal adenocarcinoma. Ann Thorac Surg 2011; 91(5):1523-30
118. Sivarasan N, Smith G. Role of aspirin in chemoprevention of esophageal adenocarcinoma: a meta-analysis. J Dig Dis 2013;14(5):222-30
119. Uotila P. The role of cyclic AMP and oxygen intermediates in the inhibition of cellular immunity in cancer. Cancer Immunol Immunother 1996;43(1):1-9
120. Jankowski J, Barr H. Improving surveillance for Barrett's oesophagus: aspECT and BOSS trials provide an evidence base. BMJ 2006; 332(7556):1512
121. Rees JR, Lao-Sirieix P, Wong A, Fitzgerald RC. Treatment for Barrett's oesophagus. Cochrane Database Syst Rev 2010(1):CD004060
122. Kastelein F, Spaander MC, Steyerberg EW, et al. Proton pump inhibitors reduce the risk of neoplastic progression in patients with Barrett's esophagus. Clin Gastroenterol Hepatol 2013;11(4):382-8
123. Miyashita T, Shah FA, Harmon JW, et al. Do proton pump inhibitors protect against cancer progression in GERD? Surg Today 2013;43(8):831-7
124. Garcia Rodriguez LA, Lagergren J, Lindblad M. Gastric acid suppression and risk of oesophageal and gastric adenocarcinoma: a nested case control study in the UK. Gut 2006;55(11):1538-44
125. Scozzari G, Trapani R, Toppino M, Morino M. Esophagogastric cancer after bariatric surgery: systematic review of the literature. Surg Obes Relat Dis 2013;9(1): 133-42
126. Kastelein F, Spaander MC, Biermann K, et al. Nonsteroidal anti-inflammatory drugs and statins have chemopreventative effects in patients with Barrett's esophagus. Gastroenterology 2011;141(6):2000-8; quiz e13-4
127. Ogunwobi OO, Beales IL. Statins inhibit proliferation and induce apoptosis in Barrett's esophageal adenocarcinoma cells. Am J Gastroenterol 2008;103(4):825-37
128. Beales IL, Hensley A, Loke Y. Reduced esophageal cancer incidence in statin users, particularly with cyclo-oxygenase inhibition. World J Gastrointest Pharmacol Ther 2013; 4(3):69-79
129. Choi SE, Perzan K, Tramontano AC, et al. Statins and aspirin for chemoprevention in Barrett's esophagus: results of a cost-effectiveness analysis. Cancer Prev Res [Phila] 2014;7(3):341-50
130. Alexandre L, Clark AB, Bhutta HY, et al. Statin use is associated with reduced risk of histologic subtypes of esophageal cancer: a nested case-control analysis. Gastroenterology 2014;146(3):661-8
131. Prasad G. Modulation of esophageal inflammation in Barrett's esophagus by omega-3 fatty acids, a double-blind placebo controlled randomized pilot study. 2012. [Epub ahead of print]
132. Rao CV, Reddy BS. Modulating effect of amount and types of dietary fat on ornithine decarboxylase, tyrosine protein kinase and prostaglandins production during colon carcinogenesis in male F344 rats. Carcinogenesis 1993;14(7):1327-33
133. Ryan AM, Reynolds JV, Healy L, et al. Enteral nutrition enriched with eicosapentaenoic acid [EPA] preserves lean body mass following esophageal cancer surgery: results of a double-blinded randomized controlled trial. Ann Surg 2009; 249(3):355-63
134. Yu C, Zhang Q, Zhang HY, et al. Targeting the intrinsic inflammatory pathway: honokiol exerts proapoptotic effects through STAT3 inhibition in transformed Barrett's cells. Am J Physiol Gastrointest Liver Physiol 2012;303(5): G561-9
135. Chung MY, Lim TG, Lee KW. Molecular mechanisms of chemopreventive phytochemicals against gastroenterological cancer development. World J Gastroenterol 2013;19(7):984-93
136. Oh TY, Lee JS, Ahn BO, et al. Oxidative stress is more important than acid in the pathogenesis of reflux oesophagitis in rats. Gut 2001;49(3):364-71
137. Jenkins GJ, D'Souza FR, Suzen SH, et al. Deoxycholic acid at neutral and acid pH, is genotoxic to oesophageal cells through the induction of ROS: the potential role of anti-oxidants in Barrett's oesophagus. Carcinogenesis 2007;28(1):136-42
138. Ibiebele TI, Hughes MC, Nagle CM, et al. Dietary antioxidants and risk of Barrett's esophagus and adenocarcinoma of the esophagus in an Australian population. Int J Cancer 2013;133(1):214-24