The esophagitis to adenocarcinoma sequence; the role of inflammation

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Esophageal adenocarcinoma (EAC) is the eighth most common cancer worldwide, and approximately 15% of patients survive 5 years. Reflux disease (GERD) and Barrett's esophagus (BE) are major risk factors for the development of EAC, and epidemiologic studies highlight a strong association with obesity. The immune, inflammatory and intracellular signaling changes resulting from chronic inflammation of the esophageal squamous epithelium are increasingly well characterized. In GERD and Barrett's, an essential role for T-cells in the initiation of inflammation in the esophagus has been identified, and a balance between T-cell responses and phenotype may play an important role in disease progression. Obesity is a chronic low-grade inflammatory state, fueled by adipose tissue derived- inflammatory mediators such as IL-6, TNF-α and leptin, representing a novel area for targeted research. Additionally, reactive oxygen species (ROS) and receptor tyrosine kinase (RTK) activation may drive progression from esophagitis to EAC, and downstream signaling pathways employed by these molecules may be important. This review will explain the diverse range of mechanisms potentially driving and maintaining inflammation within the esophagus and explore both existing and future therapeutic strategies targeting the process.

1. Introduction

1.1. The esophagitis to adenocarcinoma sequence

Barrett's esophagus (BE), characterized pathologically by specialized intestinal metaplasia (SIM), is a pre-malignant condition arising from chronic reflux disease (GERD) [1,2]. It is generally accepted that GERD, which results in chronic mucosal damage, develops as a result of a direct caustic injury to the luminal surface of the squamous epithelium, caused by the reflux of acidic gastric juices into the distal esophagus [3,4]. The damage to the surface cells is thought to stimulate a proliferative response in the underlying basal cells resulting in the characteristic basal cell hyperplasia [5]. An inflammatory response is also present with histological reports showing an inflammatory infiltrate in the squamous epithelium and chronic mucosal inflammation characterized by IL-8 release [6]. As with other chronic inflammatory conditions, esophagitis is associated with an increased risk of developing cancer- patients with esophagitis have a relative risk of 4.5 for developing EAC, this increases to 29.8 in patients who have progressed to BE [7]. It is therefore important to examine the factors driving this inflammation and to understand pathways at play, which may increase the risk of developing cancer. This may include examining the immune cells present at each stage and investigating how they promote tissue damage and drive inflammation along the progression. As such this review will focus on the role of T-cells in driving inflammation in the esophagus and the progression from esophagitis to EAC. Obesity has previously been shown to result in the establishment of a chronic low grade inflammatory environment and is known to increase the risk of BE and EAC [8]. The effect of adipose tissue derived inflammatory mediators such as IL-6, TNF-α and leptin in esophagitis, BE and EAC will be discussed, in addition to the related signaling pathways; receptor tyrosine kinases (RTK), NF-κB and STAT3 (Fig. 1). Furthermore, existing and future treatments targeting this pathological inflammation will also be discussed.

1.2. The role of T-cells in the progression from esophagitis to EAC

T-cells play an essential role in the initiation, maintenance and termination of the inflammatory response. However, their emerging role in the progression from esophagitis to EAC has become a recent focus. In a rat model of reflux disease, T cells were found to infiltrate the sub mucosal layers prior to any tissue damage and prior to any other immune cells, suggesting that these cells may play a key role in the initiation of the inflammatory response in esophagitis patients [9]. In humans, levels of the T-cell chemoattractant, RANTES are increased in esophagitis patients, allowing for the recruitment of T-cells [10]. T-cell function, as measured by PHA-induced blastogenesis, is significantly reduced in patients with esophagitis and BE [11], with BE patients showing a reduction

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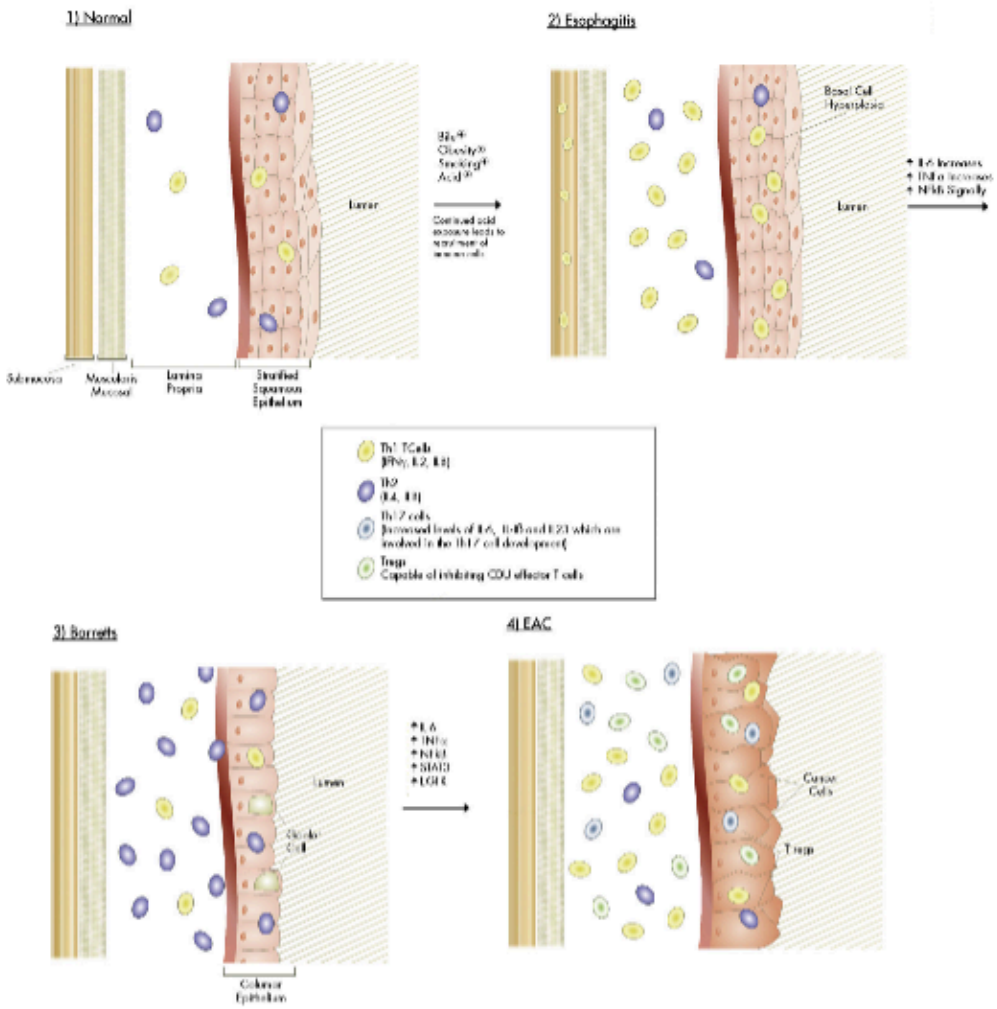

Fig. 1. The esophagitis to esophageal adenocarcinoma sequence. The continued exposure of the squamous epithelium to gastric acid and bile results in the production of inflammatory mediators such as IL-8 and IL-1β. This initiates the inflammatory response in the esophagus leading to lymphocyte recruitment and tissue damage. In esophagitis levels of IL-8, IL-1β and IFN-γ are increased indicating a pro-inflammatory response with Th1 cells predominating. The release of pro-inflammatory molecules such as IL-6, TNF-α and increased NFκB signaling are hypothesized to further drive the progression to BE, in which a predominantly humoral or Th2 response is seen. Continued increase in IL-6, TNF-α and NFκB, along with increased STAT3 and EGFR signaling are thought to promote the neoplastic conversion to EAC. Finally in EAC increased levels of Th1 cells are again seen along with increased Tregs which may dampen down the anti-tumor immune response thus providing a mechanism for immune evasion.

in systemic IL-2 production. As IL-2 is involved in clonal expansion of T-cells following activation, this suggests potential T cell dysfunction in BE [11]. However, further studies are required to elucidate the role of T cells and their function in disease progression.

$CD4^+$ T-cells can be further subdivided into subsets including Th1, Th2 and Th-17 depending on their cytokine profile. By producing IL-2, IFN-γ, and TNF-α, Th1 $CD4^+$ cells mediate tumor rejection and tissue destruction in part by driving the $CD8^+$ cytotoxic response [12]. Th2 $CD4^+$ cells produce IL-4, IL-6 and IL-10 facilitating a humoral response whilst suppressing cell-mediated immunity. The balance of these T cell responses has been suggested to play an important role in progression from oesophagitis to EAC. A recent paper by Souza et al. demonstrated that the epithelial layer was not directly damaged by the refluxed acid, but instead the acid triggered the production of chemokines and cytokines, including IL-8 and IL-1β, which mediated tissue damage [9]. In this study, reflux was induced in a rat model and at 3 days post-induction no damage to the surface epithelial cells was present, however basal cell hyperplasia was observed. Notably a lymphocytic infiltrate in the submucosa was detected by day 3 which increased in the lamina propria by week 1 and the epithelial layer only by week 3, suggesting that inflammation starts in the submucosa and progresses out towards the luminal surface. Furthermore they showed that exposure of squamous cells to acid and bile salts promote the secretion of the chemokine IL-8 and the cytokine IL-1β, which significantly enhance the rate of lymphocyte migration. This suggests that cytokine secretion by epithelial cells in response to acid exposure may initiate the inflammatory response, causing epithelial injury [9]. In humans, Fitzgerald et al. examined the cytokine profile and inflammatory cell infiltrate in both esophagitis and BE [1]. In esophagitis patients levels of IL-1β, IFN-γ and IL-8 were increased compared to controls, whereas Barrett's patients showed increased levels exclusively of IL-10 and IL-4. This suggests that while esophagitis is characterized by a pro-inflammatory cell-mediated cytokine profile, in Barrett's the predominant response appears to be an anti-inflammatory Th2 like response. This switch to a Th2 response has been proposed to drive the development of BE [13]. IL-4, which is increased in BE compared to esophagitis and normal esophageal

tissue, is known to induce the expression of MUC2 and the differentiation of epithelial cells to goblet cells in a human pulmonary mucoepidermoid cell line [14]. Similar findings in mice demonstrated that IL-4 producing $CD4^+$ T cells induce the differentiation of intestinal epithelia cells into goblet cells [15], suggesting that increased levels of IL-4 could drive metaplasia through the induction of the MUC2 gene in BE [13]. This distinct inflammatory profile in Barrett's may be an important factor in the development of EAC as it suppresses classical cell mediated anti-tumor immune responses [12]. Proinflammatory cytokines, such as IFN-γ, IL-2, IL-1β and the chemokine IL-8 have been shown to be increased in EAC compared to healthy controls and BE [16,17], indicating a further phenotypic switch to a cell mediated type 1 response. However, the role of this immune phenotypic switch in disease progression has yet to be elucidated. Further research into this area would provide information into the mechanisms of disease initiation and progression.

Histological studies have examined the expression of IL-17 along the BE to EAC sequence and demonstrated a positive association between disease progression and IL-17 levels [18]. While the source of this IL-17 was not identified, it may be due to an increase in Th-17 cells, and further studies are required to clarify this. While not studied in esophagitis and BE, Th-17 cells are increased in EAC and peripheral blood compared with healthy controls, and in advanced compared with localized disease [19,20]. Moreover, IL-6, IL-1β and IL-23, cytokines involved in Th-17 development, were shown to be present in tissue of advanced esophageal cancer [19,20]. Th-17 cells from cancer patients were shown to constitutively express CCR4 and CCR6 and their corresponding chemokines CCL22 and CCL20 were found in the tumor microenvironment in esophageal cancer patients. This suggests that the presence of cytokines and chemokines in the tumor environment could promote the differentiation and accumulation of Th-17 cells in esophageal cancer [19].

One of the principal roles of regulatory T cells (Tregs) is to prevent sustained inflammation and autoimmunity, thus preventing excess damage to healthy tissue. They act by inhibiting the activation of effector $CD4^+$ and $CD8^+$ T cells and within the tumor microenvironment this may promote tumor growth [21]. Increased levels of Tregs have been found in esophageal cancer [22] and these can suppress the proliferative activity of $CD4^+$ cells. Numerous studies have shown that tumor cells can release the chemokine CCL22, [19,21,23] which attracts Tregs to the tumor microenvironment and can promote "immune privilege", allowing tumor cells evade immune destruction [23,24]. Tregs have also been shown to correlate with patient survival, with increased levels associated with a poor prognosis in gastric and esophageal cancers [22]. While Tregs are known to promote tumor progression through the attenuation of the anti-tumor immune response, they may also control inflammation associated with tumor initiation, progression and growth [25]. The role of Tregs in esophagitis and Barrett's is less clear. Increased levels of Tregs have been identified in children with GERD [26], while decreased levels are demonstrated in the gastric cardia of adult patients with GERD [27]. There is a paucity of studies on the role of Tregs in BE, however one study reported that Tregs in BE and EAC have a higher rate of apoptosis suggesting that immune control mechanisms are compromised during disease progression [28]. The role of Tregs along the progression from esophagitis to EAC needs to be delineated, as dysfunction in Tregs may drive chronic inflammation and neoplastic conversion.

1.3. Reactive oxygen species in esophagitis and EAC

ROS are defined as oxygen containing species that can readily oxidize other molecules, they include superoxide ($O_2^{\cdot-}$), hydroxyl radicals ($HO^{\cdot}$) and hydrogen peroxide (H_2O_2). ROS are constantly generated under normal conditions in the body, for example oxidative phosphorylation in the mitochondria is a constant source of $O_2^{\cdot-}$ [29]. Another important source for ROS production comes from cells of both the innate and the adaptive immune system with increased levels of ROS associated with T-cell activation [30].

Increased levels of ROS have been reported in esophagitis and BE and are hypothesized to mediate mucosal damage in esophagitis and drive disease progression [31,32]. BE cells exposed to a low pH may be a source of endogenous ROS, causing DNA lesions and allowing for the accumulation of mutations thereby promoting carcinogenesis [33]. In progressing from GERD to EAC, levels of the anti-oxidant glutathione decrease, coupled with the accumulation of DNA lesions [34]. A recent study revealed that the oxidant generating enzyme iNOS increases from GERD to EAC, while the anti-oxidant Mn-SOD is reduced, enabling an increase in the formation of oxidative DNA lesions and potential mutations [35].

ROS are not only relevant to tumor initiation but also may promote cell growth and survival thus contributing to cancer development. For example, the platelet derived growth factor receptor (PDGFR) and the epidermal growth factor receptors (EGFR) have been shown to signal in part through ROS dependent mechanisms [36,37]. Ligand binding to these receptors results in the production of phosphatidylinositol (3,4,5)-triphosphate (PIP3) and Akt activation; a protein kinase which plays a role in cellular proliferation and inhibition of apoptosis. However, PIP3 also results in the activation of NADPH oxidase (NOX), a major source of $O_2^{\cdot-}$ which can be converted to H_2O_2 by the enzyme superoxide dismutase (SOD) [38]. Phosphatase and tensin homolog (PTEN) is a tumor suppressor protein that negatively regulates the Akt signaling pathway through the dephosphorylation and inactivation of PIP3. However PTEN is inhibited by H_2O_2 and thus H_2O_2 generation may lead to enhanced Akt signaling, enhanced cellular proliferation and inhibition of apoptosis [39]. H_2O_2 has been shown to increase cell proliferation in the BAR-T and OE33 cell lines (Barrett's and esophageal adenocarcinoma cell lines, respectively) [40,41]. Accordingly, ROS may play a role in disease progression through the promotion of tumor growth and survival and thus altering levels of oxidative species may be beneficial in treatments.

1.4. Obesity, GERD and Barrett's esophagus

Epidemiologic studies strongly link obesity, EAC and BE [42]. Obesity represents a state of chronic systemic inflammation and dysmetabolism, which is thought to play a major role in the pathogenesis of obesity-related disease [43]. Multiple mechanisms have been postulated to explain the link between obesity and BE; namely an increased rate of GERD, elevated serum pro-inflammatory adipocytokine secretions, insulin and insulin-like growth factors and increased leptin levels [44–46]. Obesity, particularly central or visceral adipose tissue (VAT) results in higher intragastric pressure creating a gastroesophageal pressure gradient, which may predispose to GERD [47,48]. Conversely, physiologic assessment reveals that the association is complex and obesity is only weakly positively associated with lower esophageal sphincter pressure during inspiration and inversely associated during expiration [47]. An effect on inflammatory pathways, fueled by VAT is therefore more plausible since obesity and VAT is associated with carcinogenesis at many tumor sites [49]. Obesity as a modifiable risk factor is therefore of great importance. The differences between visceral adipose tissue (VAT) and subcutaneous adipose tissue (SCAT) are well-established. VAT is more heavily infiltrated with inflammatory cells and more capable of producing pro-inflammatory cytokines; TNF-α, CRP and IL-6[50]. On this topic, a recently published observational study by Rubenstein et al. has been added to the discussion [51]. Interestingly, this group found an inverse relationship between the presence of gluteofemoral obesity and Barrett's esophagus when waist circumference is ad-

justed for. Consistent with this finding is that of Beasley et al. who report a trend towards lower systemic inflammation with increasing thigh subcutaneous fat in women where the inverse is seen with increasing visceral adiposity suggesting a potentially protective effect associated with thigh fat [52]. Indeed there is evidence to suggest that thigh fat has the capability to act as a type of 'sink'; examination of results from the Hoorn Study suggest that accumulation of fat in the legs is protective against disturbed glucose metabolism also [53].

1.4.1. Adipose tissue and inflammatory derived mediators

Adipocytokines exert well-established effects on glucose homeostasis and on autocrine, paracrine and endocrine signaling pathways relevant to carcinogenesis and progression [54,55]. Studies have implicated leptin, TNF-α and IL-6 in the progression of BE to EAC which is of particular interest as these are significantly increased in the obese setting [56,57]. Plasma leptin levels rise logarithmically with an increase in body weight [58]. There is no evidence to date suggesting a role for leptin in progression of esophagitis to BE, however Francois et al. demonstrated that esophageal epithelium expresses high levels of leptin receptors in both superficial and basal layers [56]. Patients with BE have significantly higher fundic leptin levels than patients with esophagits or normal epithelium [59]. Mechanistically, leptin activates signal transducer and activator of transcription (STAT) proteins -1, -3, -5 and -6, a potential mechanism of tumor progression in EAC [60]. Leptin also activates mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (EKR) and phosphatidylinositol 3-kinase (PIK3)/Akt which stimulate cell proliferation and inhibits apoptosis [61]. Leptin also stimulates esophageal adenocarcinoma growth *in vitro* by non-apoptotic mechanisms [58]. Whilst leptin is capable of binding to any one of six receptors situated throughout the central nervous system and peripheral tissues it's intracellular signaling capabilities are dependent on one isoform; the long leptin receptor or ObRb, ObRb expression is present in esophagus, stomach, small intestine, colon, liver, pancreas and gallbladder [59,62–68].

Work by our group has revealed that EAC from patients expressed the ObR and that relative expression is increased in the majority of EAC tumors compared with normal esophageal tissue [69]. Additionally, patients with higher ObR expression have significantly larger waist circumferences than those whose tumors exhibited low ObR expression, suggesting an upregulation of leptin signaling capacity with increased VAT. Furthermore, tumors with high ObR expression correlated with tumor stage, further supporting a link with disease progression [69]. Leptin has been demonstrated to stimulate esophageal cancer cell proliferation *in vitro* which is thought to occur via increased gene expression of HB-EGF and TGF-α and subsequent activation of EGFR [70].

IL-6 levels are also elevated with increasing weight, BMI, waist circumference and waist-hip ratio [71]. Li et al. have illustrated a directly proportional relationship between increased IL-6 and increased levels of Tight Junction (TJ) proteins, which are believed to increase in the early development of reflux esophagitis [72]. Zhang et al. used a series of human Barrett's epithelial cell lines to determine IL-6 mRNA expression and protein secretion/expression of p-STAT3. They reported that STAT-3 signaling was IL-6 dependent and that siRNA knockdown of STAT3 results in increased rates of apoptosis when the cells are exposed to deoxycholic acid (DCA), illustrating the anti-apoptotic and pro-survival effects of IL-6/JAK/STAT3 pathway activation in BE [73]. Dvorakova et al. demonstrated increased IL-6 and IL-6 receptor secretion from epithelial cells in BE when compared with normal duodenum and esophageal squamous epithelium. In addition, downstream of IL-6, pSTAT3, Bcl xl and Mcl-1 levels are also increased in BE [74]. The same group further demonstrated an *in vivo* induction of the IL-6/STAT3 pathway following exposure to bile acid and a low pH, which raises the possibility that refluxate working in tandem with systemic adipocytokines can further accelerate the neoplastic process [75].

TNF-α expression is present at low levels in normal squamous epithelium and increases throughout the metaplasia–dysplasia–carcinoma sequence in Barrett's esophagus [76]. It is expressed in and secreted by VAT, and TNF-α expression levels correlate with the degree of adipose tissue [77]. In BE, levels are highest within regions of intense lymphocytic infiltration and increase during the progression from metaplasia to dysplasia to carcinoma [76]. Cadherins are a family of cellular adhesion molecules, which maintain intercellular connections. TNF-α expression down-regulates expression of E-cadherin at a transcriptional level and low E-cadherin expression is observed in increasingly metaplastic tissue, providing a direct mechanism for the metastatic progression of Barrett's esophagus in an environment with high TNF-α expression [78].

1.5. Receptor tyrosine kinases (RTK) alterations in progression from BE to EAC

RTK up-regulation is a frequent occurrence in epithelial malignancies and is known to be an early event in the sequence from Barrett's to EAC, particularly during low grade dysplasia (LGD) development [79]. There is a significant increase in EGFR overexpression during the transition from high grade dysplasia (HGD) to EAC, suggesting it plays a more significant role in invasive rather than pre-invasive disease [79]. Immunohistochemical profiling of EAC specimens has revealed co-expression of at least four out of the six following RTKs; VEGFR-1, -2, -3, PDGFR-alpha, -beta and EGFR1, in 91% of specimens [80]. Transcriptomic profiling of BE-associated EAC identified potential therapeutic targets, notably an up-regulation of Axl, a RTK involved in the stimulation of cellular proliferation, during the multi-step progression from BE to EAC [81]. This was also associated with shortened median survival and antagonism of Axl significantly reduced anchorage-independent growth, invasion and migration of EAC cells [81].

Recent years have seen novel approaches to cancer therapies targeting RTKs. Constitutively active RTKs can be identified in individual tumors using RTK arrays. Gene expression profiling from esophageal and gastric tumors has been used to identify active signaling pathways, which revealed that a number of RTKs are predominantly activating the mitogen activated protein kinase (MAPK) pathway [82]. These tumors demonstrated diverse activation profiles and 65% of cases having more than two active RTKs suggesting that molecular phenotyping can inform a rational choice of targeted therapy [82].

1.6. Transcription factors; NF-κB and STAT3 signaling in Barrett's esophagus

NF-κB transcription is up-regulated along the sequence of BE to EAC with concomitant increased levels of the pro-inflammatory cytokines and transcriptional targets including IL-8 and IL-1β [83,84]. Additionally, NF-κB signaling is induced by exposure to physiological levels of the bile acid deoxycholic acid (DCA). This results in increased IL-8 transcription and IκB production resulting in a self-propagating process whereby pathway signaling is amplified [85]. Signal transducer and activator of transcription (STAT) 3 mediates the activity of cytokines involved with cancer-promoting inflammatory responses by promoting at least three of the hallmarks of cancer (proliferation, survival and angiogenesis) [86]. The canonical activation pathway involves a series of steps whereby latent circulating STAT3 is phosphorylated and translocates to the nucleus where it binds DNA and produces a transcriptional

response [87,88]. *In vitro* studies have shown that expression of phosphorylated STAT-3 is increased in cells which have undergone malignant transformation but not in non-transformed BE, suggesting that activation of the signaling pathway is a late stage development in Barrett's tumorigenesis [73]. This study demonstrated higher levels of IL-6 in the transformed Barrett's cells and that STAT3 activation in that setting is IL-6 dependent and confers a pro-survival effect [73]. These findings are consistent with previous work showing that activated pSTAT3 is present in the nuclei of dysplastic Barrett's and adenocarcinoma cells [75]. The interactions between both signaling pathways are both synergistic and occasionally antagonistic. STAT3 opposes the activation of anti-tumor immunity programs by NF-κB through inhibiting the expression of NF-κB target genes involved in Th1 innate immunity and adaptive responses to microbial infections and tumor growth [89].

1.7. Anti-inflammatory treatments in esophagitis, BE and EAC

1.7.1. Acid suppression therapy

Due to the role esophageal acid exposure plays in the development and progression of esophagitis, acid suppression therapy has become a mainstay of treatment in both esophagitis and BE. PPIs may provide good symptom control, can induce remission from reflux esophagitis for up to 5 years but rarely impact on the length of BE [90–93]. However, PPIs may have anti-inflammatory effects other than acid suppression including anti-oxidant properties, anti-apoptotic cell modulation and effects on neutrophils, epithelial cells and endothelial cells [94–97]. Whether PPIs influence the risk of EAC is controversial, but PPIs may reduce the risk of malignant transformation in BE cultures, and this is most effective in patients with pulsed as opposed to continuous acid exposure [98–101]. A recently published multicenter prospective study of 540 patients with BE demonstrated a reduced risk of neoplastic progression with PPI use both at inclusion in the study (HR 0.41, 95% CI 0.18–0.93) or during follow-up (HR 0.21, 95% CI 0.07–0.66), however length of Barrett's esophagus was unaffected [102]. Studies have shown that 20% of BE patients will continue to have pathologic reflux despite PPI use [103]. Where medical therapy fails, anti-reflux surgery can be effective, with the theoretic added value of controlled both acid and bile reflux, and we have previously reported improved control of the inflammatory milieu with surgery in a case control study of asymptomatic BE when compared with PPIs [104].

1.7.2. Targeting arachadonic acid metabolism

Aspirin is a non-steroidal anti-inflammatory drug that inhibits the cyclooxygenase enzymes, resulting in a reduction in prostaglandin levels and reduced inflammation [105]. A randomized, double blind, placebo-controlled phase two trial has revealed aspirin in high doses significantly reduces tissue concentrations of PGE_2 in patients with BE with either no dysplasia or low-grade dysplasia [106]. Selvan et al. performed an *in vitro* study demonstrating that aspirin prevents activation of proliferative and anti-apoptotic MAP kinases such as p38 and ER [72]. COX-2 is increasingly expressed when progressing from BE to EAC which provides a theoretical rationale for an inhibitor of the pathway [107]. An epidemiological study of 600,000 US adults followed for 6 years found that aspirin use was associated with a 90% reduced risk of developing esophageal cancer [107]. As COX-2 induces the production of Th2 cytokines and down regulates Th1 cytokine levels it could possibly modulate the inflammatory disease sequence [108]. By blocking COX-2, aspirin could also potentially inhibit this switch to a type-2 immune response and promote an anti-tumor Th1 response, reducing the risk of carcinogenesis [93]. The AspECT trial was specifically designed to examine the chemopreventative effect of aspirin and acid suppression therapy in BE both individually and in combination, and this will be completed in 2019 [109,110].

1.7.3. Dietary chemoprevention

Both eicosapentenoic acid (EPA) and docosahexaenoic acid (DHA) can compete with arachadonic acid for the COX enzyme and inhibit its metabolism. Much of the work to date has been carried out on colorectal cancer, however a phase 4 double blind randomised controlled trial has been commenced where examining the modulatory effect of Omega-3 Fatty Acids on Barrett's esophagus and is due for completion in 2014 [111–113].

Curcumin has been proffered as a substance with anti-cancer properties. *In vitro* studies show that it significantly abrogates DNA damage and NF-κB activation induced by bile, in fact pretreatment of OE33 cells with curcumin abolishes the ability of DCA to activate NF-κB [114]. *In vitro* studies have demonstrated the capacity of curcumin to reduce the viability of a range of esophageal cancer cell lines within 24 h of treatment [115]. Cytotoxicity is associated with an 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. Additionally, it doubles apoptotic frequency *in vivo* in Barrett's epithelial cells [114].

STAT3 is a mediator of intrinsic inflammation in BE. Honokiol (a polyphenol in herbal tea) has pro-apoptotic effects associated with the inhibition of STAT3. *In vitro*, it increases necrosis and apoptosis in transformed but not non-transformed Barretts cells, exhibiting a similar effect in adenocarcinoma cells [116]. There are a number of commercially available STAT-3 inhibitors. Whilst none have been applied to the BE/EAC setting to date, it remains a potential therapeutic target in the future.

1.7.4. Antioxidant therapy

ROS levels have been reported to be increased in esophagitis compared to healthy controls in both patients [31,32] and murine models [117,118], and are hypothesized to mediate mucosal damage and drive disease progression. Administration of a number of antioxidants have been shown to prevent mucosal damage in models of esophagitis [117–119] suggesting that anti-oxidant treatment should be considered as a therapy in the treatment of esophagitis. Epidemiological studies have suggested that high dietary intake of antioxidants is associated with a reduced risk of BE and EAC [120,121], however, results across studies have not been consistent [122] with some showing no association between antioxidant levels and BE [123]. *In vitro* studies have demonstrated that treatment with antioxidants including vitamin C and C-PTIO [2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide] can prevent DNA damage by DCA in OE33 cells [124]. Thus given the role of ROS in carcinogenesis treatment with antioxidants may provide a mechanism by which mucosal damage and DNA damage can be prevented in both esophagitis and BE respectively. While this is potentially an interesting treatment option, *in vitro* data in this area is extremely lacking and needs to be explored.

2. Conclusion

The progression from normal squamous epithelium to dysplasia, metaplasia and EAC is complex and not yet fully understood. Exposing the lower esophageal junction to acid and bile refluxate initiates a series of inflammatory changes within the esophageal mucosa. Recent evidence suggests that this triggers production of inflammatory chemokines resulting in lymphocytic tissue infiltration. Immune cell infiltration results in the production of inflammatory mediators and ROS, which are likely to mediate tissue

damage further. The presence of T cells increases within inflamed esophageal tissue and they exhibit a phenotypic change through the progression of esophagitis to BE to EAC from Th1 to Th2 and back to Th1 again. The mechanism behind this switch and the relevance is not yet fully understood and studies in this area would greatly improve on understanding of the role of adaptive immunity in disease progression. Targeting obesity-associated inflammation is also important, as signaling via STAT3, NF-κB, MAPK and EKR may enhance the pro-inflammatory and tumorigenic state. Both chemoprevention and specific anti-inflammatory approaches are being actively investigated, which may compliment or supplant the indirect approaches of current therapies targeting acid production and reflux.

Conflict of Interest

The authors declare no conflict of interest.

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