Esophageal adenocarcinoma: a review and perspectives on the mechanism of carcinogenesis and chemoprevention

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The incidence rate of esophageal adenocarcinoma (EAC) has increased sharply in the past 30 years. Many risk factors have been identified and gastroesophageal reflux disease (GERD) is the most important one. Columnar-lined esophagus, resulting from GERD, is recognized as a key precursor lesion of EAC. In this article, we review the studies on EAC in humans and animal models. We propose that the pathogenesis of EAC is mainly driven by inflammation and oxidative stress, which are augmented by iron overload. The overproduction of prostaglandin E2 and leukotriene B4 and overexpression of their receptors are believed to be major factors in exacerbating inflammation and oxidative stress. Based on this mechanistic understanding, antioxidants, inhibitors of arachidonic acid metabolism enzymes and receptor antagonists of certain eicosanoids are proposed as potential chemopreventive agents for EAC in future studies.

Esophageal adenocarcinoma (EAC) has received considerable attention in recent years because of its rapid increase in incidence. Between 1976 and 1990, the incidence rate of EAC in the United States tripled, with a yearly increase of ~10%, which was the fastest increase of all types of cancers (1). Columnar-lined esophagus (CLE, also known as Barrett’s esophagus) is a common medical condition afflicting the human esophagus and is characterized by the replacement of the squamous epithelium in the lower esophagus by columnar epithelium (2). It has been well established that CLE is the premalignant condition from which EAC arises almost exclusively. Since EAC has an extremely poor prognosis, it is of great importance to understand the pathogenesis and to develop strategies for the prevention of this deadly disease.

The classical definition of CLE is the presence of columnar epithelium in the esophagus at a length of >3 cm above the gastroesophageal junction. Three histological types of CLE have been described in the literature: (i) gastric-fundic type epithelium; (ii) gastric-junctional type epithelium; and (iii) specialized or intestinal type epithelium. Only the specialized or intestinal type CLE is susceptible to the development of EAC. Recently, intestinal type epithelium with a length of <3 cm has also been correlated with EAC (3). Therefore, the practical definition of CLE is the presence of specialized intestinal type epithelium in the esophagus, regardless of length (4).

Esophageal adenocarcinoma continues to have an extremely poor prognosis. According to a recent report using data from the Surveillance, Epidemiology and End Results Program of the National Cancer Institute, improvements in stage at diagnosis and in survival between 1973 and 1991 were minor and clinically insignificant; the overall five-year survival rate was ~10%. Stage of cancer at diagnosis was still the strongest determinant of prognosis (5). Esophageal adenocarcinoma associated with CLE had better prognosis than the one not associated with CLE (6). One out of three EAC patients died within one year after clinical diagnosis, and the median survival time was 23 ± 5 months (7). Therefore, it is very important to understand the disease process and to prevent this deadly disease at an early stage. This article discusses the mechanism of carcinogenesis and possible strategies for the prevention of this disease. For the clinical presentations and management of CLE and EAC, please refer to recent reviews (3,8).

Epidemiology, etiology and pathogenesis of esophageal adenocarcinoma

Epidemiology

Previously, EAC accounted for only ~5% of esophageal cancers in the US, while squamous cell carcinoma was the dominant type. Esophageal adenocarcinoma now accounts for >50% of esophageal cancers and afflicts ~10,000 people per year. The same situation has been found in other industrialized countries, such as the United Kingdom, Scandinavia, France, Switzerland, Australia and New Zealand (9,10). Based on 19 previous reports, the EAC risk of CLE patients varied from 1 in 46 to 1 in 441 patient-year, with a median of about 1 in 100 patient-year (11). The risk is 30–125-fold higher in CLE patients than in the normal population (12).

It is now clear that most EAC develop from CLE, which are derived from gastroesophageal reflux disease (GERD). In Western countries, GERD is a commonly seen clinical entity, with >30% of the general population experiencing its symptoms at least once every month. About 10% GERD patients will finally develop CLE (12). Eight to 20 per cent of the patients endoscopically examined for GERD and 44% of patients endoscopically examined for chronic stricture of the esophagus were diagnosed with CLE (2). One large autopsy study estimated the prevalence of CLE to be ~1 out of 80 in the US, which is 21-fold higher than that calculated from endoscopy-derived data (13). Gastroesophageal reflux disease was not always symptomatic, causing many CLE patients to be undiagnosed or misdiagnosed. Columnar-lined esophagus of a length <3 cm (8–17% of adults) was more prevalent than the classically defined CLE (14).

Abbreviations: CLE, columnar-lined esophagus; Cox, cyclooxygenase; 2-DE, two-dimensional gel electrophoresis; EAC, esophageal adenocarcinoma; EDA, esophagogastrroduodenal anastomosis; EGDA, esophagogastroduodenal anastomosis; GERD, gastroesophageal reflux disease; INOS, inducible nitric oxide synthase; Lox, lipoygenase; LT, leukotriene; LTA4H, leukotriene A4 hydrolase; NSAIDs, non-steroidal anti-inflammatory drugs; PG, prostaglandin; PLA2, phospholipase A2; ROS, reactive oxygen species.
Etiology and risk factors

Columnar-lined esophagus was originally believed to be congenital (15). More and more evidence, however, has shown that CLE is an acquired disease, although the possibility of congenital origin cannot be excluded in some rare cases (16). There is also evidence for the genetic predisposition to the development of GERD and CLE in families of CLE and EAC patients (17).

Many risk factors for the development of CLE and EAC have been described in the literature. Gastroesophageal reflux disease is the most important factor for the development of CLE and subsequent EAC. Severity of gastroesophageal reflux is related to the likeliness of developing CLE. Clinically, CLE occurs under a number of conditions that may cause gastroesophageal reflux, i.e. metatized esophagus of achalasia, scleroderma, active duodenal ulcer disease, previous gastric surgery, ill ingestion injury and Crohn’s disease (18). Spechler et al. (19) studied 25 GERD patients who had an esophageal strictured without CLE. Eleven of them developed CLE 12–50 months after the diagnosis. This study strongly suggested that GERD was the cause, rather than the result, of CLE. A recent epidemiological study conducted in Sweden showed that in patients with recurrent symptoms of reflux, the odds ratio for EAC was 7.7 as compared with healthy controls; among patients with long-standing and severe symptoms of reflux, the odds ratio was 43.5. The risk of squamous cell carcinoma, however, was not associated with reflux (20).

The prevalence of EAC increases with age, reaching a plateau by the seventh decade (21). Although the strong male predominance suggested a role of sex hormones in the etiology of EAC, antiandrogeneic treatment in prostate cancer patients did not significantly decrease the risk of EAC (22). Our study with an animal model did not suggest a strong male predominance in mice (23). Columnar-lined esophagus and EAC were more common in Caucasians than in African-Americans (24). Other risk factors included high intake of red meat and polysaturated fats, lower consumption of fruits, vegetables, fish, vitamins A, C and E, β-carotene and crude fibers (25–29), high body iron stores (30), obesity (31), cigarette smoking and alcohol consumption (32), certain medications relaxing the lower esophageal sphincter (such as calcium channel blockers, theophylline and β-agonists) (33) and immunosuppressive status (34). *Helicobacter pylori* infection was previously suggested as a risk factor. However, recent studies suggested a protective effect of some strains of *H. pylori* against EAC (35).

Pathogenesis: the inflammation–metaplasia–dysplasia–adenocarcinoma sequence

The association of GERD and CLE with EAC is firmly established. The sequence of events leading to EAC from GERD is thought to involve the development of inflammation-stimulated hyperplasia and metaplasia, followed by multifocal dysplasia, carcinoma in situ and, finally, invasive adenocarcinoma (18). Physiological gastroesophageal reflux occurs in normal people and produces neither symptoms nor histological changes in the esophagus. Only when the gastroesophageal reflux is frequent and severe may it cause symptoms or complications. Duodenogastric reflux is also a common phenomenon. In 60–70% of individuals, fasting gastric juice appeared to be stained by bile (36). However, CLE patients had higher bile acid levels in the stomach than healthy controls and GERD patients without CLE (37). A recent study employing ambulatory 24 h esophageal pH and bilirubin monitoring showed that a mixed reflux of gastric and duodenal juices is more harmful to the esophagus than gastric juice alone (38). There is synergism between duodenal contents and gastric contents in inducing CLE, although either of them alone is also damaging (39).

Since the squamous epithelium is more vulnerable to refluxate and the columnar epithelium is more resistant, replacement by columnar epithelium more or less relieves the symptoms of GERD. The most favored hypothesis for the origin of CLE is metaplasia of pluripotent stem cells in the basal cell layer upon repeated stimulation from the refluxate (40,41). At least two other possible origins of CLE have been proposed: (i) creeping substitution of columnar cells from the gastric cardia (42); and (ii) propagation of the columnar cells from the esophageal gland duct, as observed in both human patients and experimental animal models (43). Although the metaplasia hypothesis is favored, it does not exclude other possibilities. The most important intermediate marker for malignant potential in CLE is severe dysplasia. If it occurs, EAC may soon appear (44).

Studies with animal models

Animal models

Several animal models have been developed to study CLE and EAC. Surgery is the most commonly used method for induction of reflux of gastric and duodenal contents into the esophagus. Esophageal mucosal stripping with or without stimulated gastroesophageal reflux leads to replacement of squamous epithelium by columnar epithelium, but failed to produce intestinal type columnar epithelium (41,45). Duodeno-foremastach reflux (40), a pancreaticoesophageal reflux model, a bilioesophageal reflux model (41) and esophageojunostomy with or without gastrectomy and combined with or without Roux-en-Y reconstruction (47), were also reported in the literature.

Attwood et al. (48) used duodenoesophageostomy (side-to-side and end-to-side, also known as esophagoduodenal anastomosis, EDA) to induce EAC in rats (Figure 1A). The addition of 2,6-dimethylnitrosomorpholine or methyl-n-nitrosoanamine treatment to the model promoted the formation of tumors showing both squamous cell carcinoma and EAC characteristics, with nests of cells producing keratin in one area and mucin in another. Only a small percentage of tumors were pure well-differentiated EAC (49,50). A later report by the same group using the end-to-side model confirmed the previous results, and found that a high-fat diet promoted carcinogenesis (51).

Recently, the EDA rat model was adapted in our laboratory (51,52). The animals developed CLE, CLE with dysplasia and EAC at a low incidence rate (~10%), but the incidence of EAC was greatly enhanced, to 73% (8/11) at 30 weeks after surgery, when iron [50 mg Fe/kg/month, intraperitoneally (i.p.)] was administered to the animals to alleviate the postoperative anemia. Although most of the CLE appeared to develop from creeping substitution of duodenal epithelium, there were a few cases with CLE far removed from the squamocolumnar junction, and individual columnar cells interspersed in an area of squamous cells along the bottom half of the esophagus.

The EDA model and similar models, however, have inherent problems. The malabsorption of certain nutrients due to partial loss of the function of the stomach resulted in compromised
Esophageal adenocarcinoma

A.

![Diagram A](image)

B.

![Diagram B](image)

**Fig. 1.** Surgical models of esophageal adenocarcinoma (50,53). They were performed under general anesthesia (80 mg ketamine and 12 mg xylazine per kg body wt, i.p.), through an upper midline incision. (A) Esophagoduodenal anastomosis (EDA): the gastroesophageal junction is ligated flush with the stomach and the distal esophagus transected proximal to the ligature. An enterotomy is made 1 cm distal to the pylorus on the anti-mesenteric border. The distal esophagus was then anastomosed to the duodenal enterotomy with accurate mucosal to mucosal opposition. (B) Esophagogastroduodenal anastomosis (EGDA): two 1.5 cm incisions were made each on the gastroesophageal junction and the duodenum on the anti-mesenteric border, and then were anastomosed together with accurate mucosal to mucosal opposition. Care was taken not to reach the glandular stomach when the incision on the gastroesophageal junction was made.

Effects of individual constituents of gastric and duodenal juices

Hydrochloric acid and pepsin in gastric juice, as well as conjugated and unconjugated bile acids, trypsin and lyssolecithin in duodenal juice have been implicated in esophageal mucosal injury and the subsequent development of EAC. It is commonly accepted that the esophageal mucosa is relatively resistant to hydrochloric acid alone, unless it is at very high concentrations (about pH 1.0). When strong acid contacts the mucosa, especially when mixed with bile acids or pancreatic enzymes, the mucosa loses its impermeability and back infusion of hydrogen ions occur. This may also allow potential carcinogens to penetrate the mucosal barrier and act on mucosal stem cells, thereby promoting detachment of the surface cells from the epithelium (58).

The role of gastric acid in the development of EAC is still controversial in the literature. Many clinical studies have shown that acid suppression controls symptoms of GORD, promotes healing of GORD, prevents complications of GORD, even induced shortening and shrinking of CLE and regeneration of squamous islands (59,60). On the other hand, antireflux surgery did not induce CLE regression and eliminate the risk of EAC (61). In one report, patients on acid suppressive treatment had a higher risk for EAC than those who did not receive treatment (62). In a study with surgical animal models, gastric acid protected the animals from developing EAC induced by the reflux of duodenal contents and carcinogen exposure (63), although such a protective effect was not observed in the absence of a carcinogen (64). Continuous acid exposure on an *ex vivo* organ culture system with human
CLE biopsy induced differentiation and reduced proliferation, whereas short pulses of acid increased proliferation (65). Bile acid pulses enhanced cell proliferation via the protein kinase C pathway. Combination with acid significantly inhibited the bile acid-induced CLE hyperproliferation (66). We may postulate that gastric acid may be a conditional accomplice of bile acids, and its effect depends upon the mode of action.

It is known that reflux of duodenal juice induces both EAC and squamous cell carcinoma in rats, and the carcinogenesis is enhanced by nitrosamines (40). Apart from lysolecithin and trypsin in the duodenal juice, bile acids are the most likely noxious agents and have received much research attention. Bile acids themselves are not carcinogenic, but the secondary bile acids (degradation products of the primary bile acids by bacteria), lithocholic acid and deoxyxylithocholic acid, are possible co-carcinogens (67). Their effects seem to depend on the surrounding pH and the conjugation status (68). When the pH is between 2 and 7, especially between 3 and 5, unionized and lipophilic bile acids can move through the esophageal mucosal barrier into the mucosal cells and then ionize to cause cellular damage (38). Acid-suppressive therapy may actually promote mucosal damage by bile acids through regulating the pH environment (38). Unconjugated bile acids are known to be cancer promoters at neutral pH (69), whereas conjugated bile acids are harmful at low pH levels. Although bile acids have long been suspected to undergo nitrosation and serve as precursors of carcinogens (70), a recent study on the duodenal juice of rat after esophageojunostomy with mass spectrometry failed to detect any N-nitrosotaurocholic acid or N-nitroso-glycocholic acid (71).

Bile acids can activate ornithine decarboxylase (72) and cyclooxygenase 2 (Cox2) in an AP1-dependent mechanism through the protein kinase C pathway (73). These and other actions of bile acids may also cause oxidative stress, as reflected in the activation of oxidative stress-related genes (74). Further investigation is needed to clarify the roles of gastric acid and bile acids in the pathogenesis of CLE and EAC concerning: (i) the interaction between gastric acid and bile acids; (ii) the relative importance of conjugated and unconjugated bile acids; and (iii) the downstream events upon stimulation by gastric acid and bile acids, and the relevant pathways.

Effects of iron supplementation and oxidative stress
Since chronic inflammation, such as GERD, is known to induce persistent oxidative stress, it would be reasonable to postulate that oxidative stress is a driving force for adenocarcinogenesis. Wetscher et al. (75,76) used a short-term duodenogastrooesophageal reflux model to induce esophagitis by ligating the distal end of the duodenum and performing cardiomyotomy. Injection of superoxide dismutase or buthionine sulfoximine reduced the severity of esophagitis. These results suggested that esophageal adenocarcinogenesis may also be an inflammation- and oxidative stress-driven process.

Using both the EDA model and the EGDA model, we further demonstrated in long-term studies that iron supplementation promoted the formation of EAC in rats (Table I). Iron was found deposited in the esophagus after surgery and iron administration. Overexpression of inducible nitric oxide synthase (iNOS) and the presence of nitrotyrosine were observed and were correlated with inflammation and cell proliferation (50,51). Esophageal iron overload might result from transient increase of blood iron after i.p. injection and the overexpression of transferrin receptor in the premalignant CLE cells. Oxidative damage to DNA, protein and lipid in the esophagus was significantly higher in the treated rats than in the non-operated control. CLE cells were believed to be the target cells of oxidative damage because they overexpressed heme oxygenase 1 and metallothionein, both are known to be responsive to oxidative stress (52). Supplementation of vitamin E in the diet (10-fold) maintained the normal plasma level of α-tocopherol in the EDA rats and inhibited esophageal adenocarcinogenesis (77).

Cellular and molecular changes in human CLE and EAC
In esophageal adenocarcinogenesis, progressive accumulation of genetic and epigenetic aberrations is believed to lead to one or more clones with malignant potential. Many genetic and epigenetic abnormalities, such as gene mutation, gene deletion, loss of heterozygosity, aberrant methylation and aberrant gene expression, have been reported in human EAC and CLE. Many genes have been reported to be involved in some cases. These include: (i) apoptosis-related genes, such as bcl-2, Fas, Fas ligand, cathepsin B and DcR3 (78–81); (ii) cell cycle-related genes, such as cyclin D1, p16, p15, p21 and p27 (82–85); (iii) tumor suppressor genes, such as Rb, APC, MCC, DCC and DPC4 (86,87); (iv) oncogenes and related genes, such as H-ras, K-ras, c-ras, c-src, c-jun, c-fos, c-myc, c-erbB2, osteopontin and cathepsin L (88–90); and (v) growth factors and their receptors, differentiation markers, cell surface markers and various intracellular enzymes. For recent reviews please refer to Altorki et al. (12), Lieshout et al. (91), Casson (92), Fitzgerald and Triadafilopoulos (93), Beer and Stoner (94) and Rustgi (95).

p53 is the most extensively studied gene in EAC. The reported p53 mutation data showed that ~50% of EAC had p53 mutations, whereas in CLE and CLE with dysplasia, p53 mutation was less frequent (12,96). Most of these mutations were located in exons 5, 7 and 8, but prominent hotspots were not found in CLE and EAC. Transition was the most common type of mutation, and most were G:C to A:T occurring frequently at the CpG dinucleotides. The G:C to A:T transition at the CpG dinucleotide may be the result of deamination of 5-methylcytosine either occurring spontaneously, or caused by nitric oxide produced by overexpressed iNOS during CLE and EAC (97). p53 mutation analysis may be useful for early diagnosis, prognosis and treatment of CLE and EAC (98). More than 50% of CLE-associated EAC and CLE with high-grade dysplasia, <50% of CLE with low-grade dysplasia and a small percentage of the CLE patients without dysplasia exhibited p53 overexpression by immunohistochemistry (86,99). Some late-stage EAC patients exhibited serum autoantibody (100). Similar to the human situation, loss of p53 function predisposed animals to the formation of EAC (23). In an E1A/E1B transgenic mouse model, adenocarcinoma developed at the squamocolumnar junction in the foregut with p53 disruption (101).

Recently, Barrett et al. (102) dissected out the clonal ordering of neoplastic lineages in human EAC. p53 gene mutation, p16 gene mutation, non-random loss of heterozygosity, p16 methylation and ploidy were analyzed, and many intermediate clones with cancer-developing tendency were detected, suggesting multiple pathways leading to EAC. Some of these clones persisted and developed into EAC, whereas others had no progression or were delayed in their progression. Such
studies of the genetic events in carcinogenesis may improve our understanding of CLE and EAC, help in identifying high-risk patients and thus lead to more efficient strategies for prevention and treatment.

Several new techniques have been used for identification of molecular changes in esophageal adenocarcinogenesis. Genome-scale microsatellite analysis and comparative genomic hybridization have been used for locating potential tumor suppressor genes and oncogenes (87,103). Restriction landmark genomic scanning two-dimensional gel electrophoresis has successfully identified two amplified genes in human EAC, cathepsin B (80) and GATA-4 (104). cDNA expression array and two-dimensional gel electrophoresis (2-DE) of protein are two effective methods for the detection of differentially expressed genes and proteins. Combining the RNA and protein expression data to comprehensively profile both transcriptional and post-transcriptional changes in cells and tissues is particularly appealing (105,106). Our group recently identified several genes overexpressed in EAC of the EGDA rat model with the cDNA expression array and protein 2-DE combined with mass spectrometry. Both methods showed overexpression of glucose-regulated protein 94, a heat shock protein, in tumor tissues. Western blotting and RT–PCR further confirmed the results in both rat and human tissues. Immunohistochemical staining revealed that its expression in goblet cells increased in CLE and further increased in EAC (107). Soldes et al. (108) found low-level expression of heat shock protein 27 in human CLE and EAC samples with 2-DE of protein. With further technical improvements, more and more interesting results would be expected, and may fundamentally change the scope and pace of the research on EAC in the future.

Abnormal arachidonic acid metabolism and oxidative stress as key factors for esophageal adenocarcinogenesis

Although the cause–effect relationship between GERD, CLE and EAC is clear, the molecular process starting from chronic inflammation, to genetic and epigenetic changes, and finally to cancer is still not well defined. Chronic inflammation is regulated by many factors, among which arachidonic acid metabolites and reactive oxygen species (ROS) are of particular importance.

Arachidonic acid metabolism in chronic inflammation and carcinogenesis

The correlation between arachidonic acid metabolism and carcinogenesis is suggested by studies on non-steroidal anti-inflammatory drugs (NSAIDs), which target different arachidonic acid-metabolizing enzymes. Long-term use of NSAIDs in rheumatic patients is related to reduced risk of various human cancers, including esophageal cancer (109,110). NSAIDs exert chemopreventive effects against cancer formation at various sites, including the upper digestive tract (111,112).

Non-steroidal anti-inflammatory drugs are believed to exert their chemopreventive effects mainly by inhibiting arachidonic acid metabolism, although other mechanisms have also been suggested (113,114). A large body of evidence showed that phospholipase A2 (PLA2) inhibitors, Cox inhibitors and lipoxygenase (Lox) inhibitors suppressed cancer formation or cancer cell growth in an arachidonic acid metabolite-dependent manner (115–118). Homologous disruption of either the Cox1 or Cox2 gene reduced polypl formation in Min/+ mice (119). Cox inhibitor and Lox inhibitor synergistically inhibited NNK-induced lung cancer in A/J mice (120).

Among the eicosanoids produced from arachidonic acid, prostaglandin E2 (PGE2 from the Cox pathway) and leukotriene B4 (LTB4 from the 5-Lox pathway) are the most potent inflammatory mediators and most extensively studied. In the presence of inflammation, human squamous esophageal mucosa produced relatively large amounts of eicosanoids via the Cox and 5-Lox pathways, and lesser amounts of metabolites from the 12-Lox pathway. These changes were correlated with the degree of tissue damage. Treatment with omeprazole for GERD reduced the production of PGE2 and LTB4 (121,122).
**Cox pathway**

Cox2 was overexpressed in human EAC (97). Cox2 expression was also increased significantly in biopsied CLE tissue in response to pulses of acid or bile acids in an *ex vivo* organ culture system, and this effect was attenuated by a selective Cox2 inhibitor (123). Celecoxib, a Cox2 inhibitor, induced apoptosis in human EAC cell lines (124).

Eicosanoids are unstable and their activities are normally restricted to the cells in the immediate vicinity that express the specific receptors. PGE2 receptors (EP1, EP2, EP3, and EP4) were all found to be expressed in goblet cells of rat small intestine by *in situ* hybridization (125). Binding of PGE2 to its receptors initiates the signaling mediated by receptor subtype-specific G proteins and respective changes of second messengers (cAMP, Ca$^{2+}$, and inositol phosphates), and induces tumor growth and metastasis (126). Chronic intestinal inflammation may increase the expression of some EP receptors (127). EP receptors are believed to be important for carcinogenesis. In EP1-knockout mice, the azoxymethane-induced aberrant crypt foci formation decreased significantly (128). Specific EP1 antagonists have been shown to be protective against azoxymethane-induced aberrant crypt foci in both wild-type mice and Min$^{+/−}$ mice (129).

Similar to the human situation (122), Cox2 was overexpressed and PGE2 overproduced in the CLE and EAC tissues of the rat surgical model, and EP4 receptor was expressed in the goblet cells. It is possible that, in our surgical animal models, activation of the Cox2 pathway stimulates the production of PGE2, which may then bind to the EP receptors on goblet cells to induce hyperproliferation. Meanwhile, the squamous epithelium in the esophagus was shedded off due to inflammatory erosion. These combined events facilitate the creeping substitution of squamous epithelium by the columnar epithelium. Mucin-producing nature of the rat EAC may result from hyperproliferation of EP receptor-expressing goblet cells upon stimulation by PGE2.

**Lox pathways**

Among the three Lox pathways, the 5-Lox pathway is often closely related to chronic inflammation and carcinogenesis. LTB4, the eicosanoid produced from the 5-Lox and leukotriene A4 hydrolase (LTA4H) pathway, induces recruitment and activation of neutrophils, monocytes, eosinophils and lymphocytes, and stimulates the production of a number of pro-inflammatory cytokines and mediators, indicating its ability to augment and prolong tissue inflammation (130). It stimulated the proliferation of a colon cancer cell line (131). In human GERD and CLE, the levels of LTβ4 and PGE2 in the biopsy esophageal mucosa were significantly higher than normal (132). LTβ4 has two distinct receptors, with BLT1 mainly involved in chemotaxis, whereas BLT2 in degranulation and superoxide production. Similar to the PG signaling pathways, Gi protein, cAMP increase and Ca$^{2+}$ influx are the second messengers of the LTβ4-mediated signaling (132,133). Studies using transgenic mice and specific antagonists have confirmed the involvement of BLT1 in inflammation (134,135).

Inhibition of 5-Lox suppressed inflammation (136), cell growth (137) and tumor formation in animal models (120,138). Disruption of the 5-Lox pathway by gene knockout attenuated acute inflammation (139,140). A specific LTA4H inhibitor, bestatin, induced apoptosis in some human non-small-cell lung cancer cell lines (141), inhibited carcigen-induced stomach tumors in Wistar rats (142), and had been used as adjuvant treatment for various human cancers, including leukemia, malignant melanoma, lung cancer, stomach cancer, bladder cancer, head and neck cancer, and esophageal cancer (143).

In our previous study using protein 2-DE, LTA4H was identified as an overexpressed protein (~3-fold) in rat EAC tumor as compared with normal tissue. The result was confirmed in both rat and human tissues by immunohistochemistry. We observed overproduction of LTB4 in rat EAC tumors, as well as in human GERD and CLE biopsy samples. We also observed overexpression of 5-Lox in rat and human EAC tumor cells, and the infiltrating mononuclear cells in inflammatory region and tumors. These preliminary results suggest an important role of the 5-Lox pathway in esophageal adenocarcinogenesis.

12-Lox and 15-Lox pathways are less studied than the 5-Lox pathway. 12-Lox metabolites promoted cancer cell proliferation, metastasis and angiogenesis (144). 12-Lox inhibitors suppressed cancer formation in animal models (145). 15-Lox, however, seemed to be protective against inflammation and carcinogenesis (146).

**Oxidative stress in chronic inflammation and carcinogenesis**

Oxidative damage has been proposed as a possible mechanism for human GERD and possibly also CLE (147). Wetscher et al. (148) measured the amount of ROS (by chemiluminescence), expressed in the EAC tumor cells, and the in oxidative damage can be exacerbated by exogenous iron overload, as revealed in our animal model studies. Meanwhile, local inflammation regulates iron distribution *in vivo*. Lactoferrin produced by macrophages removes iron from transferrin in acidic inflammatory environments, and deliver the iron to macrophages. Macrophages of the reticuloendothelial system can incorporate more iron into apoferritin, which is synthetized in larger amounts during inflammation, and then transfer the iron by migration to the site of inflammation (152). Iron deposited in the reticuloendothelial system is poorly reused. Existing inflammation recruits iron from the blood to the site of inflammation, where iron promotes oxidative damage and thus exacerbates the inflammation. The extent of inflammation may not be correlated with the general iron nutritional status, but is very likely correlated with the iron...
Esophageal adenocarcinoma

**Fig. 2.** Proposed histopathological and molecular events leading to esophageal adenocarcinogenesis. Gastroduodenoesophageal reflux causes irritation and inflammation. Reactive oxygen species and inflammatory mediators (especially arachidonic acid metabolites) are proposed to induce genetic and epigenetic changes leading to CLE, CLE with dysplasia, and EAC. This hypothesis, developed on the basis of the animal models, may also be applicable to humans.

- **Local irritation and inflammation**
  - Gastroesophageal reflux
  - Gastroesophageal reflux disease (GERD)
  - Gastric contents
  - Gastric acid
  - Bile acids
  - Digestive enzymes

- **Arachidonic Acid Metabolites**
  - Prostaglandins
  - Leukotrienes (LT)
  - other inflammatory mediators

- **Genetic and Epigenetic Changes**
  - Genetic instability and hyperproliferation
  - Altered gene expression and cell cycle control
  - Gene mutation and allelic loss
  - Apoptosis
  - Protein amplification

**Concluding remarks: mechanism of esophageal adenocarcinogenesis and its prevention**

Based on our understanding of esophageal adenocarcinogenesis, a hypothesis for a chronic inflammation-driven mechanism is proposed (Figure 2). The pathogenesis starts from GERD in humans or reflux-inducing surgery in animal models. Gastric acid, bile acids and digestive enzymes induce irritation and inflammation in the esophagus. The squamous epithelium of the esophagus responds with inflammatory cell infiltration, hyperkeratinization, basal cell hyperproliferation and even sloughing or ulceration of the epithelium. Two categories of inflammatory mediators produced by the inflammatory cells in the esophagus, eicosanoids (such as PGE\(_2\) and LTB\(_4\)) and ROS, are of particular importance. They exert feedforward effects on the inflammatory cells and direct effects on cells expressing respective receptors, such as the goblet cells expressing EP receptors for PGE\(_2\). Reactive oxygen species, on the other hand, may cause DNA strand breaks, DNA base modification, lipid peroxidation and protein oxidation. In the surgical animal models, both eicosanoids and ROS may stimulate the growth of columnar epithelium at the squamocolumnar junction, to induce creeping substitution of the squamous epithelium in the esophagus by columnar epithelium. In human patients with GERD, metaplasia of the stem cells in the esophagus or creeping substitution by the intestinalized cardiac epithelium may be induced. Persistent stimulation on the columnar cells by these inflammatory mediators will result in a series of genetic and epigenetic changes, such as gene mutation and allelic loss, genomic instability, gene amplification, hyperproliferation, altered gene expression and cell cycle control, and altered apoptosis. Morphologically we observe a pathological progression starting from GERD to CLE, CLE with dysplasia, and finally EAC.

This hypothesis may help us identify targets and design strategies for the chemoprevention of EAC. It is important that the intervention should be administered before the pivotal genetic or epigenetic changes occur. Beer and Stoner (94) have proposed several chemopreventive strategies for the chemoprevention of EAC, such as cell proliferation inhibitors, inhibitors of metabolic activation of procarcinogens, inducers of protective mechanisms, certain dietary factors and NSAIDs. Based on our hypothesis, we propose several categories of chemopreventive agents for future studies: (i) inhibitors of the key enzymes of arachidonic acid-metabolizing pathways, e.g., PLA\(_2\), 5-Lox, LTA\(_4\)H and 12-Lox; (ii) antagonists of certain eicosanoid receptors and (iii) antioxidants. Combination of agents with different acting mechanisms may have synergistic, additive or compensatory effects, e.g., a combination of an NSAID and an antioxidant, a combination of a Cox2 inhibitor and a 5-Lox inhibitor, a combination of a Cox2 inhibitor and a gastroprotective PGE agonist, or a cocktail of several agents.

Potential problems related to the use of these agents for chemoprevention of esophageal adenocarcinogenesis should be considered. The relative contribution of eicosanoids to inflammatory responses may vary among species, strains, tissues and cells (163). The arachidonic acid metabolism pathways are closely related to each other in terms of mechanisms of action and substrate preference; therefore, enzyme specificity may be a problem. Unexpected results may be observed due to interactions among the pathways, i.e. blocking of one pathway may activate another pathway (164). Feedback pathways may be functioning in regulating the effects of eicosanoids, i.e. LTB\(_4\) activates peroxisome proliferator activated receptor \(\alpha\) to induce cellular responses, but also induces transcription of its own catabolic enzymes (165). 15d-PGJ\(_2\), a Cox metabolite derived from PGD\(_2\), can suppress the expression of Cox2 (166).

As for lifestyle and dietary recommendations for prevention of EAC, a primary approach may include (i) avoid over-eating and obesity; (ii) prevent iron overload by decreasing red meat intake and avoiding iron-enriched food; (iii) consume more fiber-containing and plant-based food; (iv) stop smoking and drinking.
reduce the consumption of alcohol. Individuals with GERD should consult with gastroenterologists to treat GERD and use esophageal sphincter-relaxing medication with caution.

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