Review

Cancer Research

Pathology, Chemoprevention, and Preclinical Models for Target Validation in Barrett Esophagus

Aleksandra M. Urbanska[1], Selvarangan Ponnazhagan[2], and Masoud Mozafari[3,4,5]

Abstract

Despite esophageal adenocarcinoma (EAC) being the most widespread among gastrointestinal cancers, with an 11-fold increase in the risk of cancer for patients with Barrett esophagus (BE), its prognosis is still poor. There is a critical need to better perceive the biology of cancer progression and identification of specific targets that are the hallmark of BE's progression. This review explores the established animal models of BE, including genetic, surgical and nonsurgical approaches, potential chemoprevention targets, and the reasoning behind their applications to prevent Barrett-related EAC. The key methodological features in the design feasibility of relevant studies are also discussed. *Cancer Res; 78(14); 3747–54. ©2018 AACR.*

Introduction

Barrett esophagus: Definition and epidemiology

The esophagus is a muscular tube, which joins the proximally located pharynx to the distally located stomach. The esophagus is a very well assembled system with many efficient and complex protective tools against reflux injury. This includes the antireflux barriers (restricting the gastric chyme from accessing the esophagus), the luminal clearance mechanisms (ensuring a short period of contact between the refluxate and the esophageal epithelium), and the tissue resistance (which minimizes the damage to the esophageal epithelium; ref. 1). If the gastroesophageal reflux is persistent, it causes chronic mucosal injury and inflammation. Many tumors of the digestive system arise under such conditions, including esophageal adenocarcinoma [EAC; from Barrett esophagus (BE)], gastric cancer (from *Helicobacter pylori*–associated gastritis), hepatocellular cancer (from viral hepatitis), and colon cancer (from inflammatory bowel disease).

BE is a preneoplastic lesion in which the normal, stratified esophageal squamous epithelium is substituted with intestinal-type columnar epithelium. Such a conversion from esophageal to intestinal-type cells is known as intestinal metaplasia (IM). IM originates at the junction of the distal esophagus and the gastric cardia, that is, the gastroesophageal junction (GEJ). BE is considered to be the precursor to EAC and progresses from metaplasia to low-grade dysplasia (LGD) and high-grade dysplasia (HGD), which can end with invasive adenocarcinoma. Figure 1 depicts anatomy and physiology of the human and murine upper gastrointestinal tract.

The epidemiology of esophageal cancers in the United States has undergone a major change since 1975, when 75% of all cases were squamous cell cancer (SCC) affecting primarily the middle portion of the esophagus. Today, the rate of SCC has fallen slightly while that of EAC near the GEJ has risen dramatically (2). In 1975, EAC affected 4 people per million, in 2001, this rate escalated to 23 people per million, making it the fastest-growing cancer in the United States (3). The dramatic increase in the disease incidence is not due to an overdiagnosis; in fact, EAC mortality has increased in parallel with incidence (2). EAC is distinguished by six prominent characteristics: increasing occurrence, male prevalence, lack of preventive measures, opportunities for early detection, challenging surgical therapy and care, and poor prognosis (4, 5).

BE and EAC

The possibility of EAC in patients with BE is low, with 0.12% affected annually in unselected populations and 0.26% in patients with HGD (6). However, this corresponds to an 11-fold increase in the risk of cancer for patients with BE as opposed to those without (7). Thus, 1 of 200 patients with BE will acquire esophageal cancer every year (8). BE is observed in 5% to 15% of patients who are searching for medical care for chronic heartburn (9). However, due to the prevalent accessibility of acid-suppressant medications, numerous patients with reflux symptoms choose to not be subjected to an expensive endoscopy unless their symptoms are persistent or refractory to medical therapy. The pathogenesis of BE is likely a two-step process. The first step involves the transformation of normal esophageal squamous mucosa to a simple columnar epithelium called cardiac mucosa. This occurs in response to chronic injury produced by chronic episodes of gastric juice refluxing onto the squamous mucosa. The change from squamous to cardiac mucosa likely occurs relatively quickly, within a few years, while the second step, the development of goblet cells indicative of IM, proceeds slowly, probably over 5 to 10 years (10). Because BE is a precursor of EAC, screening is an attractive strategy to decrease cancer deaths from EAC. Unfortunately, recent guidelines from the American

[1]Division of Digestive and Liver Diseases, Department of Medicine, Columbia University Medical Center, New York, New York. [2]Department of Pathology, The University of Alabama at Birmingham, Birmingham, Alabama. [3]Bioengineering Research Group, Nanotechnology and Advanced Materials Department, Materials and Energy Research Center (MERC), Tehran, Iran. [4]Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran. [5]Department of Tissue Engineering and Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran.

Corresponding Author: Masoud Mozafari, Materials and Energy Research Center (MERC) and Iran University of Medical Sciences, Tehran, Iran. Phone: 98-912-6490679; Fax: 98-263-6280033 ×477; E-mail: mozafari.masoud@gmail.com

doi: 10.1158/0008-5472.CAN-18-0206

Published OnlineFirst June 29, 2018; DOI: 10.1158/0008-5472.CAN-18-0206

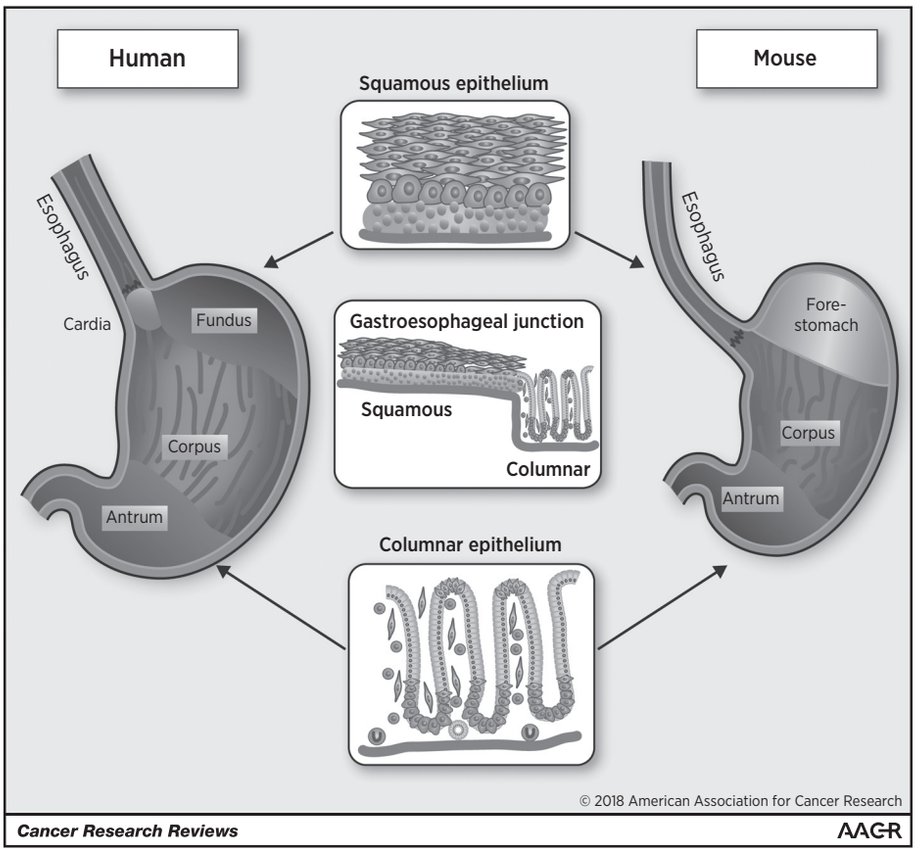

Figure 1.
Anatomy and physiology of the human and murine upper gastrointestinal tract. The esophagus, lined by stratified squamous epithelium, in humans joins the cardia region of the stomach lined by columnar epithelium at the GEJ, whereas in the mouse, it joins the stomach at the intersection of the forestomach, lined by squamous epithelium, and the corpus, lined by columnar epithelium. The GEJ shows a transition from the squamous to columnar epithelium.

Gastroenterological Association (AGA) do not recommend endoscopic screening for patients with reflux symptoms due to its cost (8). Moreover, proper diagnosis is complex and varies in different countries. For instance, in the United Kingdom, the British Society of Gastroenterology does not consider IM a required parameter (11). The complexity of proper diagnosis arises from the differences in the distinction between LGD and HGD within the BE crypts (12). The comprehensive list of known and available biomarkers of BE is included here (13).

The purpose of this review is to present a comprehensive list of available animal models used to study BE as well as discuss the current status with the literature overview of the major chemoprevention options for BE.

Risk factors

In order to prevent any disorder, it is of primary importance to understand its risk factors, and in the case of esophageal cancer, every type has different risk factors. Some of the greatest origins for EACs arise from a history of gastroesophageal reflux disease (GERD; refs. 14, 15): BE, regurgitation, obesity (16, 17), dietary factors such as low in vitamin D intake (18) and antioxidant content (19), lifestyle, genetic predisposition (20, 21), and smoking (22–24). Moreover, bulimia nervosa, the eating disorder, could be a possible risk factor in the pathogenesis of EAC (25). The declining *Helicobacter pylori* prevalence in Western countries is associated with higher rates of BE and EAC (26) as well as cardia cancer, which share features of both, esophageal and gastric cancers (27).

Diagnosis

Clinical challenges in proper identification of patients with BE include finding cost-effective and improving the diagnostic potential of endoscopic screening. Thus far, it has neither been shown that screening for BE improves mortality from adenocarcinoma, nor is the process cost effective (28). According to AGA, an endoscopic screening is recommended for patients with several risk factors related to EAC, such as old age, male sex, white race, chronic GERD, hiatal hernia, elevated body mass index, and intra-abdominal distribution of fat; ref. 8). Guidelines for diagnosing BE include columnar lining measurement, specifically that there is at least a 1 cm threshold of columnar lining above the GEJ. In addition, patients with BE require minimum of 8 biopsies. It is also proposed that endoscopists should utilize the Prague classification to describe what is seen in the Barrett segment (29). A recent study evaluated cost effectiveness of screening patients with GERD for BE with a minimally invasive cell sampling device called Cytosponge (30). The screening was found to be cost effective. There is a 5-year survival in 83% to 90% of the cases for EAC, if the tumor is identified at an early stage whereas a dismal 10% to 15% 5-year survival exists for those with late-stage cancers (28). Luckily, the development of carcinogenesis is a prolonged one that offers abundant occasions for intervention.

Treatment

During an evolution of many years, a gradual shift occurs from LGD to HGD, intramucosal cancer, and lethal disease. The obligation of the endoscopist is to properly identify each of these

Published OnlineFirst June 29, 2018; DOI: 10.1158/0008-5472.CAN-18-0206

stages and assign appropriate therapy. Endoscopic resection with either endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD) is the most accurate method for pathologic diagnosis and staging (31). Endoscopic resection such as EMR or ESD allows the removal of large specimens that include mucosa and submucosa suitable for predictive staging. Different studies have shown a clear benefit for shorter segments (up to 4 to 5 cm) or noncircumferential BE treated with stepwise EMR (32). Despite the fact that BE with dysplasia is many times more common in the West, only two reports on ESD have been published compared with many on EMR and ablation. Ablation technique such as endoscopic radiofrequency ablation (RFA) of dysplastic BE combined with proton pump inhibitor therapy is frequently used for blocking the advancement of dysplastic BE to EAC. Additional ablation modalities include antigen-presenting cell (APC), photodynamic therapy, cryoablation, and multipolar electrocoagulation. A 2013 U.S. Multicenter Consortium conducted study on 592 patients with BE treated with RFA from 2003 through 2011 and measured the rate of recurrence, described as the occurrence of IM or dysplasia after complete remission of IM (CRIM) in surveillance biopsies (33). It was found that 56% of patients were in complete remission after 24 months and 33% had disease relapse within the next 2 years. A recent Markov analysis by Hur and colleagues suggests that RFA is cost effective in preventing the progression of HGD to cancer compared with surveillance (34). They suggest a role for ablation in confirmed, stable, and multifocal LGD. The role for ablation in nondysplastic BE was unclear.

Chemoprevention and Its Challenges

Chemoprevention is a form of a clinical population prevention, proposed at diminishing the probability of disease advancement in recognized high-risk individuals by the use of pharmaceutical agents (35). In other words, it is the use of specific preventive drugs to stop cancerous cells from forming, growing, or recurring. Prescribing chemotherapy to a patient is one of the most difficult conundrums for medical practitioners. While chemotherapy can be beneficial, it can only be successful in certain settings and once animal studies have truly been exhausted to validate the efficacy of the pharmaceutical agents.

The challenges of increasing cancer prevention are numerous and daunting. The increasing rates of patients with BE indicate that the attitude toward chemoprevention needs to be re-examined. While tremendous efforts have been devoted to studying genetic and molecular changes in BE and EAC, little has translated so far into clinical practice. Identification of a highly specific and sensitive biomarker, which it purports to detect, would aid in the identification of early disease progression. However, the process of biomarkers evaluation is analogous to the process in therapeutic drug studies; it is complex, demanding, and time consuming. Table 1 summarizes chemopreventive agents currently used for BE, and their effects, limitations, and modes of action.

Animal Models to Study BE and EAC

There are a large number of cell lines to study BE, with the most commonly used ones derived from epithelium of patients with BE or EAC. They include CP-D, BAR-T-cell lines from Barrett epithelial cells and OE33, OE19, FLO-1, and SKGT4 from the EAC cell line. The presence of bile salts and their composition can be tested in such cell lines to mimic the gastroesophageal refluxate of a BE patient. Various bile salts, acids, different pHs, pulsatile exposures, and incubation times are being optimized for the most optimal to the clinical conditions in a number of studies (36–39). However, further discussion of these studies is beyond the scope of this review.

Establishment of animal models

The main challenge in studying the progression from neoplastic lesion to EAC in animals is the lack of true preclinical models that can express the evolutionary dynamics of Barrett cell populations. Mouse models differ from humans in lacking gastric reflux and gastric juice production, having a forestomach, and possessing variability in diet composition and microbiome composition. These differences present technical limitations in the development of a robust animal model that can truly mimic the human condition. From computational models, which offer a high degree of tractability, enable researchers to both, understand data and to acquire intuitions and hypotheses for neoplastic progression. From tissue culture models, which involve squamous cell lines, BE cell lines and EAC cell lines, we can learn about the microenvironment of the cells in two-dimensional conditions. However, neither of these approaches is foolproof and recapitulates the disease model of interest. For instance, BIC-1, SEG-1, and TE-7 cell lines have been recently disputed to not truly represent EAC tissue culture (40). Moreover, while three-dimensional organotypic cultures of BE might overcome many issues associated with two-dimensional cell lines, they do not fully represent a true microenvironment of the gastrointestinal tract. Mice have a histologically comparable esophagus to rats but, dissimilar to rats, mice can be easily genetically modified. Thus, mice can offer a superior model for studying the molecular modifications leading to the development of Barrett metaplasia. Using mice, it is beneficial to evaluate whether metaplasia of the proximal stomach, stimulated by conditional, localized mechanical destruction of the squamous epithelium (e.g., by gavage), can be abided and influence to neoplasia, with or without genetic lesions involved in BE. Below, we have highlighted some of the mouse models currently being used for the study of BE.

Rodent models

Genetic mouse models: p63-null mouse model. The transcription factor p63 is necessary for epidermal lineage commitment, epidermal differentiation, cell adhesion, and basement membrane formation (41). This factor is part of a family that comprises two structurally associated proteins, p53 and p73. p63 knockout mice have considerable defects in their limb and craniofacial development, including a remarkable lack of stratified epithelia (42, 43). p63-null embryos have idiopathic metaplastic changes together in the esophagus and proximal stomach, as two typically squamous tissues in mouse models (44). Although the mouse upper gut is generally lined by squamous epithelium from the mouth to the middle of stomach, p63-null embryos instead demonstrate a well-built columnar epithelium (45). Conversion of the stratified squamous esophageal epithelium to a columnar, intestinal-like epithelium, via a metaplasia is a key feature occurring in BE pathology.

The McKeon group has recently reported that BE originates from a minor population of nonesophageal cells leftover from early development (44). Furthermore, they assume that the initial spread of these cells derives not from genetic alterations but from competitive interactions between cell lineages, driven by

Published OnlineFirst June 29, 2018; DOI: 10.1158/0008-5472.CAN-18-0206

Table 1. Summary of drug classes and their mode of action for BE

Group	Trade name	Mode of action	Chemopreventive effects/study limitations	Route	Ref
Proton pump inhibitors (PPI)	Omeprazole (Prilosec, Omesec), lansoprazole (Prevacid), rabeprazole (Aciphex), pantoprazole (Protonix), esomeprazole (Nexium)	-Irreversible blocking of the hydrogen/potassium adenosine triphosphatase enzyme system (the gastric proton pump) of the gastric parietal cells	-Patients on PPIs had significant decrease in proliferating cell nuclear antigen in biopsies of Barrett metaplasia -Usage of PPIs for >2–3 years had no association with the risk of EAC and/or HGD in BE patients -In patients on PPIs for 1–13 years, no regression in overall length of Barrett metaplasia	Oral, intravenous	(67–73)
H2 receptor antagonists (H2RA)	Cimetidine (Tagamet), ranitidine (Zantac), famotidine (Pepcidine, Pepcid, Gaster), nizatidine (Axid)	-Blocking action of histamine on parietal cells in the stomach, reducing the production of acid by the cells -Have effect on the cytochrome P450 enzyme system	-No observed lowered risk of neoplastic progression in patients with BE [study excluded prevalent high-grade dysplasia; cohort consisted of 1,466 patients with a mean age of 61 $\pm$ 13 years; patients had a predominance of male sex (76.7%) and Caucasian race (96.6%)]	Oral, intravenous	(74, 75)
Nonsteroidal anti-inflammatory drugs (NSAID)	Acetylsalicylic acid (asprin), diclofenac (Voltaren), diflunisal (Dolobid), etodolac (Lodine), ibuprofen (Motrin), indomethacin (Indocin), ketoprofen (Orudis), ketorolac (Toradol), nabumetone (Relafen), naproxen (Aleve, Naprosyn), oxaprozin (Daypro), piroxicam (Feldene), salsalate (Amigesic), sulindac (Clinoril), tolmetin (Tolectin), celecoxib (Celebrex), rofecoxib (Vioxx), meloxicam (Mobic), valdecoxib (withdrawn)	-Competitive reversible inhibition of the activity of both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), and thereby the synthesis of prostaglandins and thromboxanes obtained from arachidonic acid (itself derived from the cellular phospholipid bilayer by phospholipase A2)	-Low-dose aspirin and nonaspirin COX inhibitors associated with a reduced risk of BE neoplasia; meta-analysis -Regular use of aspirin or NSAIDs was associated with a decreased risk of BE -A reduced risk of BE was not observed in patients administered with aspirin and 23 nonaspirin NSAIDs (small sample size) -Administration of 200 mg of celecoxib twice daily for 48 weeks of treatment does not appear to prevent progression of Barrett dysplasia to cancer	Oral	(76–83)
Statins	Simvastatin (Zocor), lovastatin (Altoprev, Mevacor), atorvastatin (Lipitor), fluvastatin (Lescol), pravastatin (Pravachol), rosuvastatin (Crestor)	-Competitive inhibition of HMG CoA reductase, the rate-limiting step in cholesterol biosynthesis. Small molecules occupy a portion of the binding site of HMG CoA, blocking access of this substrate to the active site on the enzyme	-Significantly lower incidence of adenocarcinoma risk in BE patients; meta-analysis of 11 studies (no adjustment for BMI or smoking; limited categorization of duration and dose relationship) -Significant (28%) reduction in the risk of EAC among BE patients; meta-analysis of 13 studies (limited info about different doses of statins; recall bias)	Oral	(84–88)
Ornithine decarboxylase (ODC) inhibitors	Eflornithine (α-difluoromethylornithine) caffeic acid phenethyl ester (CAPE)	-Blocking the activity of the enzyme ornithine decarboxylase belonging to the polyamie biosynthetic pathway	-Troglitazone caused G_1 cell-cycle arrest and reduced ODC activity in TE-7 cells but not in TE-1 cells. Inhibition by PPAR-gamma ligands of growth of EAC cells may be due to induction of apoptosis, G_1 cell-cycle arrest and reduction of ODC activity (the effect of PPARγ antagonists in low concentrations was not clear)	Oral	(89–93)
Diet-derived chemopreventive agents	Folate, curcumin, genistein, tea catechins, anthocyanins ellagitannins	-Modulation of the expression of proteins correlated with proliferation, apoptosis, inflammation, angiogenesis, and both cyclooxygenase and lipoxygenase paths of arachidonic acid metabolism -Cellular targets include kinases, telomerase, cyclooxygenase-2, triggers of apoptosis, and transcription factors AP1 and nuclear factor κB	A 100 μg/day increment in dietary folate intake reduced the estimate risk of esophageal cancer by 12%; meta-analysis of 19 studies (results could be affected by recall bias and selection bias, none of the studies analyzed total folate intake, comprising folate from the diet along with folate from supplements; the analysis used pooled data; individual data were not available)	Oral	(94)

Published OnlineFirst June 29, 2018; DOI: 10.1158/0008-5472.CAN-18-0206

opportunity. This mouse model is used to indicate the progress of a Barrett-like metaplasia in embryonic mice from precursor cells. While the developmental model serves as a good tool to address future questions as to why obscure residual embryonic cells of BE origin persist postnatally, it cannot serve as a useful tool for studying chemoprevention.

L2-IL1β mouse model. In humans, high levels of IL1β can promote chronic inflammation and carcinogenesis (46, 47). Carriers of IL1β polymorphisms (IL1β-511T and IL1β-31C) correlated with increased systemic levels of the cytokine have an increased risk of gastric cancer, and targeted overexpression of IL1β in the stomach of mice causes gastric inflammation and cancer (48, 49). Similar findings have been reported in BE, where genetic polymorphisms of IL1RA, the endogenous IL1 receptor antagonist, have been associated with higher systemic IL1β levels and to a higher risk of BE among patients with GERD (50). Raised levels of IL1β and IL8 have also been reported in biopsies of BE compared with squamous esophageal mucosa (51).

Over time, L2-IL1β mice develop esophageal hyperplasia and metaplastic BE lesions that resemble human BE in gene expression profile. The L2-IL1β mouse model provides insights into the early pathogenesis of BE and proposes a molecular basis for an emerging distinct concept concerning the cell of origin of BE and EAC. In a recent study, we used IL1β mice to show that hypergastrinemia increases proliferation and expansion of Barrett-like esophagus and accelerates a progression from BE to EAC using $CCK2R^+$ progenitor cells (52). In addition to IL1β, metaplasia can be accelerated in this mouse model by feeding the mice 0.2% deoxycholic acid, an unconjugated bile acid that is a key component of the duodenal refluxate that promotes BE in GERD (53). While both genetic mouse models, p63 null and L2-IL1β, support the hypothesis that BE arises from progenitor cells in the cardia, with the L2-IL1β mouse model, the mice survive to adulthood and develop progression to EAC.

p27 knockout model. Because, in human studies, most Barrett-associated adenocarcinomas are lacking tumor suppressor gene *p27*, p27 knockout mice were used to conduct a study where animals were exposed to a carcinogen, methylbenzylnitrosamine, following induction of the gastroduodenal–esophageal reflux (54). As expected, the highest incidence of EAC was observed in p27 knockout mice. This useful mouse model could serve as an example in cultivating techniques to prevent malignant transformation of BE; however, due to a small mouse size and associated postsurgical complications, their utility is limited.

Transitional basal cells at the squamous-columnar junction model. While no mouse model truly mimics BE in terms of the presence of intestinal goblet cells, a mouse model provided evidence that the distinct basal progenitor cells ($p63^+KRT5^+KRT7^+$), in the newly identified transitional zone in the epithelium of the upper GI tract, are the cells of origin for multilayered epithelium and BE (55). The study showed that basal cells ($p63^+KRT5^+$) in the transitional epithelium served as progenitors for $KRT7^+$ BE-like epithelium in mice. The ($Lgr5^+$) cardia mucosa consistently did not contribute to the transitional epithelium in Lrg5CreER;R26LacZ mice. Using lineage tracing, it was revealed that the columnar epithelium lining the $p63^{-/-}$ esophagus, forestomach and squamocolumnar junction is derived from p63 promoter active basal progenitors.

Surgical mouse models. In addition to genetic engineering approaches, mice serve as a tool in reflux-inducing surgeries. A mouse surgical reflux model to evaluate Barrett metaplasia and esophageal carcinogenesis that summarizes both the histologic and molecular changes prominent in human Barrett metaplasia has been developed by inducing acid reflux after following an end-to-side esophagojejunostomy (EJ; ref. 56). As early as 13 weeks after EJ, esophagitis with esophageal erosions and squamous epithelial thickening with basal cell layer hyperplasia has been observed. Within 34 weeks, columnar intestinal-type metaplasia looking like that of human BE was detected in 1 of 6 animals with erosive esophagitis. The metaplasia occurred with the presence of goblet cells. By 13 months following surgery, just 1 of 13 animals had esophageal IM. No adenocarcinoma or squamous cell carcinomas were detected. Gastroesophageal reflux was surgically induced in wild-type (WT), p53A135V transgenic, and INK4a/$Arf^{+/-}$ mice but it did not produce EAC (57). On the other hand, total gastrectomy resulting in jejunoesophageal reflux was performed in WT, p53-knockout ($Trp53^{-/-}$), or APC-mutated ($APC^{Min/+}$) mice to show that the loss of Trp53, but not APC, results in the incidence of cancer and loss of either Trp53 or APC leads to the development of columnar metaplasia. Unfortunately, due to the short life span of p53-knockout mice, application of such a model is still limited (58).

Rat model. In 1962, the first attempt to produce esophagitis in rats was performed using EJ, which yielded a high incidence rate of pancreatic reflux and slightly lower incidence of bile reflux (59). This was achieved by performing a combination of transplantation of the bile duct to a jejunal loop, gastrectomy, and transplantation of the bile duct to jejunum and diversion of pancreatic secretions. There were no metaplastic or neoplastic changes; however, the healing of traumatized squamous epithelium with glandular replacement has been observed shortly after (60). The occurrence of glandular islands of heterotopia and ectopia in the rat stomach after repeated trauma by open gastrostomy was present.

Rats were also used to model the reflux through the pylorus or through an end-gastrojejunostomy (61). While no cancers were detected in any of the animals with gastrotomy, gastric adenocarcinoma developed in 41%. The follow-up study was devised to conclude whether esophageal cancer could be provoked by a reflux of gastroduodenal contents (62). Though no neoplasia was perceived in the control rats, the experimental rats with duodeno-forestomach reflux and duodeno-glandular-forestomach reflux developed adenocarcinomas in the lower esophagus. Rats following the EJ were administered MF-tricyclic (10 mg/kg/day) and sulindac (30 mg/kg/day), resulting in lower incidence of EAC in the MF-tricyclic + sulindac groups than in the control group and lower degree of inflammation in both groups ($P < 0.001$) showing that both selective and nonselective COX-2 inhibitors might have chemopreventive potential in BE (63). In another study, rats following the EJ were out on diets containing 1% ursodeoxycholic acid (Urso) and 0.3% acetylsalicylic acid (asprin). Drug combination reduced the risk of adenocarcinoma in animals with reflux (in the combination group, 26% of animals developed esophageal cancer vs. 62% in the control group), decreased proliferation of EAC cells while no significant difference was observed in the risk of EAC in the groups given Urso alone or asprin alone when compared with control (64).

Published OnlineFirst June 29, 2018; DOI: 10.1158/0008-5472.CAN-18-0206

While the surgical rat model has offered valuable understanding into the histologic pathogenesis of Barrett metaplasia, it has not been used to look into the fundamental molecular mechanisms involved in Barrett pathogenesis due to limitations in genetic engineering.

Large animal models

Because rodents do not possess a natural reflux, drugs such as proton pump inhibitors cannot be tested readily in these models. Therefore, surgical intervention of EJ provides a true model with a chronic reflux esophagitis. Only surgically modified animals can serve as a part of the clinical background. While experiments on the canine model represent one of the best models as it truly produces reflux and leads to an IM, since the 1970s (65) and 1980s (66) the studies have fallen out of use due to numerous bans at higher education institutions.

Concluding Remarks and Future Perspectives

The occurrence of EAC is increasing in Western countries. BE is the only identified precursor of EAC. While one of the most common predisposing factors to BE is the presence of gastroesophageal acid reflux, most patients presenting with EAC report no history of acid reflux. Hence, these asymptomatic patients would not have undergone endoscopy, with a possibility of BE remaining undiagnosed during its early stages. Preemptive approaches for high-grade dysplasia and BE, such as RFA and photodynamic therapy, have achieved dramatic results; however, there are reports of recurrences in the long run. Therefore, the main challenges in the field remain identifying (i) the origin of BE, (ii) a single, reliable risk factor for BE development to aid diagnosis, (iii) uniformity in diagnostic criteria, and (iv) therapeutic drug development for BE and EAC. Identification of chemopreventive agents depends greatly on epidemiologic and pathologic data procured from human patients, animal models, and cell cultures. As enumerated in this article, multiple chemopreventive and therapeutic drugs are being used for BE and EAC; however, an effective course of action can only be taken after reliable diagnosis with histologic and molecular markers. With increasing knowledge and understanding of the pathology and progression of BE, its therapy is promising in the near future.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Received March 1, 2018; revised April 13, 2018; accepted May 22, 2018; published first June 29, 2018.

References

1. Li Y, Martin RC 2nd. Reflux injury of esophageal mucosa: experimental studies in animal models of esophagitis, Barrett's esophagus and esophageal adenocarcinoma. Dis Esophagus 2007;20:372–8.
2. Pohl H, Welch HG. The role of overdiagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence. J Natl Cancer Inst 2005;97:142–6.
3. Hoffman MH, CD. 2013 Esophageal Cancer on the Rise. <http://www.webmd.com/cancer/features/esophageal-cancer-rise>.
4. Crane SJ, Locke GR 3rd, Harmsen WS, Zinsmeister AR, Romero Y, Talley NJ. Survival trends in patients with gastric and esophageal adenocarcinomas: a population-based study. Mayo Clin Proc 2008;83:1087–94.
5. Lagergren J, Lagergren P. Recent developments in esophageal adenocarcinoma. CA Cancer J Clin 2013;63:232–48.
6. Hvid-Jensen F, Pedersen L, Drewes AM, Sorensen HT, Funch-Jensen P. Incidence of adenocarcinoma among patients with Barrett's esophagus. N Engl J Med 2011;365:1375–83.
7. Ronkainen J, Aro P, Storskrubb T, Johansson SE, Lind T, Bolling-Sternevald E, et al. Prevalence of Barrett's esophagus in the general population: an endoscopic study. Gastroenterology 2005;129:1825–31.
8. Spechler SJ, Sharma P, Souza RF, Inadomi JM, Shaheen NJ. American Gastroenterological Association medical position statement on the management of Barrett's esophagus. Gastroenterology 2011;140:1084–91.
9. Shaheen NJ, Richter JE. Barrett's oesophagus. Lancet 2009;373:850–61.
10. Oberg S, Johansson J, Wenner J, Walther B. Metaplastic columnar mucosa in the cervical esophagus after esophagectomy. Ann Surg 2002;235:338–45.
11. Sampliner RE. Updated guidelines for the diagnosis, surveillance, and therapy of Barrett's esophagus. Am J Gastroenterol 2002;97:1888–95.
12. Ibrahim NB. ACP. Best Practice No 155. Guidelines for handling oesophageal biopsies and resection specimens and their reporting. J Clin Pathol 2000;53:89–94.
13. Fouad YM, Mostafa I, Yehia R, El-Khayat H. Biomarkers of Barrett's esophagus. World J Gastrointest Pathophysiol 2014;5:450–6.
14. Parasa S, Sharma P. Complications of gastro-oesophageal reflux disease. Best Pract Res Clin Gastroenterol 2013;27:433–42.
15. Odze RD, Maley CC. Neoplasia without dysplasia: lessons from Barrett esophagus and other tubal gut neoplasms. Arch Pathol Lab Med 2010;134:896–906.
16. Solaymani-Dodaran M, Logan RF, West J, Card T, Coupland C. Risk of oesophageal cancer in Barrett's oesophagus and gastro-oesophageal reflux. Gut 2004;53:1070–4.
17. Chak A, Falk G, Grady WM, Kinnard M, Elston R, Mittal S, et al. Assessment of familiality, obesity, and other risk factors for early age of cancer diagnosis in adenocarcinomas of the esophagus and gastroesophageal junction. Am J Gastroenterol 2009;104:1913–21.
18. Meeker S, Seamons A, Paik J, Treuting PM, Brabb T, Grady WM, et al. Increased dietary vitamin D suppresses MAPK signaling, colitis, and colon cancer. Cancer Res 2014;74:4398–408.
19. Murphy SJ, Anderson LA, Ferguson HR, Johnston BT, Watson PR, McGuigan J, et al. Dietary antioxidant and mineral intake in humans is associated with reduced risk of esophageal adenocarcinoma but not reflux esophagitis or Barrett's esophagus. J Nutr 2010;140:1757–63.
20. Chak A, Lee T, Kinnard MF, Brock W, Faulx A, Willis J, et al. Familial aggregation of Barrett's oesophagus, oesophageal adenocarcinoma, and oesophagogastric junctional adenocarcinoma in Caucasian adults. Gut 2002;51:323–8.
21. Robertson EV, Jankowski JA. Genetics of gastroesophageal cancer: paradigms, paradoxes, and prognostic utility. Am J Gastroenterol 2008;103:443–9.
22. Cook MB, Kamangar F, Whiteman DC, Freedman ND, Gammon MD, Bernstein L, et al. Cigarette smoking and adenocarcinomas of the esophagus and esophagogastric junction: a pooled analysis from the international BEACON consortium. J Natl Cancer Inst 2010;102:1344–53.
23. Lagergren J, Bergstrom R, Lindgren A, Nyren O. Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma. N Engl J Med 1999;340:825–31.
24. Cook MB, Greenwood DC, Hardie LJ, Wild CP, Forman D. A systematic review and meta-analysis of the risk of increasing adiposity on Barrett's esophagus. Am J Gastroenterol 2008;103:292–300.
25. Shinohara ET, Swisher-McClure S, Husson M, Sun W, Metz JM. Esophageal cancer in a young woman with bulimia nervosa: a case report. J Med Case Rep 2007;1:160.
26. Rokkas T, Pistiolas D, Sechopoulos P, Robotis I, Margantinis G. Relationship between Helicobacter pylori infection and esophageal neoplasia: a meta-analysis. Clin Gastroenterol Hepatol 2007;5:1413–7, 7 e1–2.
27. Kamangar F, Dawsey SM, Blaser MJ, Perez-Perez GI, Pietinen P, Newschaffer CJ, et al. Opposing risks of gastric cardia and noncardia gastric

adenocarcinomas associated with Helicobacter pylori seropositivity. J Natl Cancer Inst 2006;98:1445–52.
28. Sharma P, McQuaid K, Dent J, Fennerty MB, Sampliner R, Spechler S, et al. A critical review of the diagnosis and management of Barrett's esophagus: the AGA Chicago Workshop. Gastroenterology 2004;127:310–30.
29. Falk GW. Updated guidelines for diagnosing and managing Barrett esophagus. Gastroenterol Hepatol (N Y) 2016;12:449–51.
30. Heberle CR, Omidvari AH, Ali A, Kroep S, Kong CY, Inadomi JM, et al. Cost effectiveness of screening patients with gastroesophageal reflux disease for Barrett's Esophagus with a minimally invasive cell sampling device. Clin Gastroenterol Hepatol 2017;15:1397–404e7.
31. Moss A, Bourke MJ, Hourigan LF, Gupta S, Williams SJ, Tran K, et al. Endoscopic resection for Barrett's high-grade dysplasia and early esophageal adenocarcinoma: an essential staging procedure with long-term therapeutic benefit. Am J Gastroenterol 2010;105:1276–83.
32. Pouw RE, Seewald S, Gondrie JJ, Deprez PH, Piessevaux H, Pohl H, et al. Stepwise radical endoscopic resection for eradication of Barrett's oesophagus with early neoplasia in a cohort of 169 patients. Gut 2010;59:1169–77.
33. Gupta M, Iyer PG, Lutzke L, Gorospe EC, Abrams JA, Falk GW, et al. Recurrence of esophageal intestinal metaplasia after endoscopic mucosal resection and radiofrequency ablation of Barrett's esophagus: results from a US Multicenter Consortium. Gastroenterology 2013;145:79–86e1.
34. Hur C, Choi SE, Rubenstein JH, Kong CY, Nishioka NS, Provenzale DT, et al. The cost effectiveness of radiofrequency ablation for Barrett's esophagus. Gastroenterology 2012;143:567–75.
35. Potter JD. The failure of cancer chemoprevention. Carcinogenesis 2014;35:974–82.
36. Bajpai M, Liu J, Geng X, Souza RF, Amenta PS, Das KM. Repeated exposure to acid and bile selectively induces colonic phenotype expression in a heterogeneous Barrett's epithelial cell line. Lab Invest 2008;88:643–51.
37. Das KM, Kong Y, Bajpai M, Kulkarni D, Geng X, Mishra P, et al. Transformation of benign Barrett's epithelium by repeated acid and bile exposure over 65 weeks: a novel in vitro model. Int J Cancer 2011;128:274–82.
38. Burnat G, Rau T, Elshimi E, Hahn EG, Konturek PC. Bile acids induce overexpression of homeobox gene CDX-2 and vascular endothelial growth factor (VEGF) in human Barrett's esophageal mucosa and adenocarcinoma cell line. Scand J Gastroenterol 2007;42:1460–5.
39. Feagins LA, Zhang HY, Hormi-Carver K, Quinones MH, Thomas D, Zhang X, et al. Acid has antiproliferative effects in nonneoplastic Barrett's epithelial cells. Am J Gastroenterol 2007;102:10–20.
40. Alvarez H, Koorstra JB, Hong SM, Boonstra JJ, Dinjens WN, Foratiere AA, et al. Establishment and characterization of a bona fide Barrett esophagus-associated adenocarcinoma cell line. Cancer Biol Ther 2008;7:1753–5.
41. Koster MI. p63 in skin development and ectodermal dysplasias. J Invest Dermatol 2010;130:2352–8.
42. Yang A, Schweitzer R, Sun D, Kaghad M, Walker N, Bronson RT, et al. p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development. Nature 1999;398:714–8.
43. Mills AA, Zheng B, Wang XJ, Vogel H, Roop DR, Bradley A. p63 is a p53 homologue required for limb and epidermal morphogenesis. Nature 1999;398:708–13.
44. Wang X, Ouyang H, Yamamoto Y, Kumar PA, Wei TS, Dagher R, et al. Residual embryonic cells as precursors of a Barrett's-like metaplasia. Cell 2011;145:1023–35.
45. von Holzen U, Enders GH. A surprise cell of origin for Barrett's esophagus. Cancer Biol Ther 2012;13:588–91.
46. Figueiredo C, Machado JC, Pharoah P, Seruca R, Sousa S, Carvalho R, et al. Helicobacter pylori and interleukin 1 genotyping: an opportunity to identify high-risk individuals for gastric carcinoma. J Natl Cancer Inst 2002;94:1680–7.
47. El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, et al. The role of interleukin-1 polymorphisms in the pathogenesis of gastric cancer. Nature 2001;412:99.
48. Tu S, Bhagat G, Cui G, Takaishi S, Kurt-Jones EA, Rickman B, et al. Overexpression of interleukin-1beta induces gastric inflammation and cancer and mobilizes myeloid-derived suppressor cells in mice. Cancer Cell 2008;14:408–19.
49. Fox JG, Wang TC. Inflammation, atrophy, and gastric cancer. J Clin Invest 2007;117:60–9.
50. Gough MD, Ackroyd R, Majeed AW, Bird NC. Prediction of malignant potential in reflux disease: are cytokine polymorphisms important? Am J Gastroenterol 2005;100:1012–8.
51. Fitzgerald RC, Abdalla S, Onwuegbusi BA, Sirieix P, Saeed IT, Burnham WR, et al. Inflammatory gradient in Barrett's oesophagus: implications for disease complications. Gut 2002;51:316–22.
52. Lee Y, Urbanska AM, Hayakawa Y, Wang H, Au AS, Luna AM, et al. Gastrin stimulates a cholecystokinin-2-receptor-expressing cardia progenitor cell and promotes progression of Barrett's-like esophagus. Oncotarget 2017;8:203–14.
53. Guy NC, Garewal H, Holubec H, Bernstein H, Payne CM, Bernstein C, et al. A novel dietary-related model of esophagitis and Barrett's esophagus, a premalignant lesion. Nutr Cancer 2007;59:217–27.
54. Ellis FH Jr, Xu X, Kulke MH, LoCicero J 3rd, Loda M. Malignant transformation of the esophageal mucosa is enhanced in p27 knockout mice. J Thorac Cardiovasc Surg 2001;122:809–14.
55. Jiang M, Li H, Zhang Y, Yang Y, Lu R, Liu K, et al. Transitional basal cells at the squamous-columnar junction generate Barrett's oesophagus. Nature 2017;550:529–33.
56. Pham TH, Genta RM, Spechler SJ, Souza RF, Wang DH. Development and characterization of a surgical mouse model of reflux esophagitis and Barrett's esophagus. J Gastrointest Surg 2014;18:234–40; discussion 40–1.
57. Hao J, Liu B, Yang CS, Chen X. Gastroesophageal reflux leads to esophageal cancer in a surgical model with mice. BMC Gastroenterol 2009;9:59.
58. Fein M, Peters JH, Baril N, McGarvey M, Chandrasoma P, Shibata D, et al. Loss of function of Trp53, but not Apc, leads to the development of esophageal adenocarcinoma in mice with jejunoesophageal reflux. J Surg Res 1999;83:48–55.
59. Lambert R. Relative importance of biliary and pancreatic secretions in the genesis of esophagitis in rats. Am J Dig Dis 1962;7:1026–33.
60. Wong J, Finckh ES. Heterotopia and ectopia of gastric epithelium produced by mucosal wounding in the rat. Gastroenterology 1971;60:279–87.
61. Miwa K, Hasegawa H, Fujimura T, Matsumoto H, Miyata R, Kosaka T, et al. Duodenal reflux through the pylorus induces gastric adenocarcinoma in the rat. Carcinogenesis 1992;13:2313–6.
62. Miwa K, Segawa M, Takano Y, Matsumoto H, Sahara H, Yagi M, et al. Induction of oesophageal and forestomach carcinomas in rats by reflux of duodenal contents. Br J Cancer 1994;70:185–9.
63. Buttar NS, Wang KK, Leontovich O, Westcott JY, Pacifico RJ, Anderson MA, et al. Chemoprevention of esophageal adenocarcinoma by COX-2 inhibitors in an animal model of Barrett's esophagus. Gastroenterology 2002;122:1101–12.
64. Rizvi S, Demars CJ, Comba A, Gainullin VG, Rizvi Z, Almada LL, et al. Combinatorial chemoprevention reveals a novel smoothened-independent role of GLI1 in esophageal carcinogenesis. Cancer Res 2010;70:6787–96.
65. Bremner CG, Lynch VP, Ellis FH Jr. Barrett's esophagus: congenital or acquired? An experimental study of esophageal mucosal regeneration in the dog. Surgery 1970;68:209–16.
66. Gillen P, Keeling P, Byrne PJ, West AB, Hennessy TP. Experimental columnar metaplasia in the canine oesophagus. Br J Surg 1988;75:113–5.
67. Armstrong D. Intravenous proton pump inhibitor therapy: a rationale for use. Rev Gastroenterol Disord 2005;5 Suppl 2:S18–30.
68. Cooper BT, Chapman W, Neumann CS, Gearty JC. Continuous treatment of Barrett's oesophagus patients with proton pump inhibitors up to 13 years: observations on regression and cancer incidence. Aliment Pharmacol Ther 2006;23:727–33.
69. Kastelein F, Spaander MC, Steyerberg EW, Biermann K, Valkhoff VE, Kuipers EJ, et al. Proton pump inhibitors reduce the risk of neoplastic progression in patients with Barrett's esophagus. Clin Gastroenterol Hepatol 2013;11:382–8.
70. Luk CP, Parsons R, Lee YP, Hughes JD. Proton pump inhibitor-associated hypomagnesemia: what do FDA data tell us? Ann Pharmacother 2013;47:773–80.
71. Ouatu-Lascar R, Fitzgerald RC, Triadafilopoulos G. Differentiation and proliferation in Barrett's esophagus and the effects of acid suppression. Gastroenterology 1999;117:327–35.
72. Peters FT, Ganesh S, Kuipers EJ, Sluiter WJ, Karrenbeld A, de Jager-Krikken A, et al. Effect of elimination of acid reflux on epithelial cell proliferative activity of Barrett esophagus. Scand J Gastroenterol 2000;35:1238–44.

Published OnlineFirst June 29, 2018; DOI: 10.1158/0008-5472.CAN-18-0206

73. Hu Q, Sun TT, Hong J, Fang JY, Xiong H, Meltzer SJ. Proton pump inhibitors do not reduce the risk of esophageal adenocarcinoma in patients with Barrett's Esophagus: a systematic review and meta-analysis. PLoS One 2017;12:e0169691.
74. Sigterman KE, van Pinxteren B, Bonis PA, Lau J, Numans ME. Short-term treatment with proton pump inhibitors, H2-receptor antagonists and prokinetics for gastro-oesophageal reflux disease-like symptoms and endoscopy negative reflux disease. Cochrane Database Syst Rev 2013;5: CD002095.
75. Thota PN, Hajifathalian K, Benjamin T, Runkana A, Lopez R, Sanaka MR. Lack of incremental effect of histamine receptor antagonists over proton pump inhibitors on the risk of neoplastic progression in patients with Barrett's esophagus: a cohort study. J Dig Dis 2017;18:143–50.
76. Knights KM, Mangoni AA, Miners JO. Defining the COX inhibitor selectivity of NSAIDs: implications for understanding toxicity. Expert Rev Clin Pharmacol 2010;3:769–76.
77. Tsibouris P, Vlachou E, Isaacs PE. Role of chemoprophylaxis with either NSAIDs or statins in patients with Barrett's esophagus. World J Gastrointest Pharmacol Ther 2014;5:27–39.
78. Tsibouris P, Hendrickse MT, Isaacs PE. Daily use of non-steroidal anti-inflammatory drugs is less frequent in patients with Barrett's oesophagus who develop an oesophageal adenocarcinoma. Aliment Pharmacol Ther 2004;20:645–55.
79. Ofman JJ, Maclean CH, Straus WL, Morton SC, Berger ML, Roth EA, et al. Meta-analysis of dyspepsia and nonsteroidal antiinflammatory drugs. Arthritis Rheum 2003;49:508–18.
80. Zhang S, Zhang XQ, Ding XW, Yang RK, Huang SL, Kastelein F, et al. Cyclooxygenase inhibitors use is associated with reduced risk of esophageal adenocarcinoma in patients with Barrett's esophagus: a meta-analysis. Br J Cancer 2014;110:2378–88.
81. Schneider JL, Zhao WK, Corley DA. Aspirin and nonsteroidal anti-inflammatory drug use and the risk of Barrett's esophagus. Dig Dis Sci 2015;60:436–43.
82. Khalaf N, Nguyen T, Ramsey D, El-Serag HB. Nonsteroidal anti-inflammatory drugs and the risk of Barrett's esophagus. Clin Gastroenterol Hepatol 2014;12:1832–9e6.
83. Heath EI, Canto MI, Piantadosi S, Montgomery E, Weinstein WM, Herman JG, et al. Secondary chemoprevention of Barrett's esophagus with celecoxib: results of a randomized trial. J Natl Cancer Inst 2007;99:545–57.
84. Istvan ES, Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. Science 2001;292:1160–4.
85. Singh S, Singh AG, Singh PP, Murad MH, Iyer PG. Statins are associated with reduced risk of esophageal cancer, particularly in patients with Barrett's esophagus: a systematic review and meta-analysis. Clin Gastroenterol Hepatol 2013;11:620–9.
86. Uhrinova S, Uhrin D, Powers H, Watt K, Zheleva D, Fischer P, et al. Structure of free MDM2 N-terminal domain reveals conformational adjustments that accompany p53-binding. J Mol Biol 2005;350:587–98.
87. Fujii T, Nakabayashi T, Hashimoto S, Kuwano H. Statin use and risk of gastroduodenal ulcer and reflux esophagitis. Hepatogastroenterology 2009;56:641–4.
88. Beales IL, Hensley A, Loke Y. Reduced esophageal cancer incidence in statin users, particularly with cyclo-oxygenase inhibition. World J Gastrointest Pharmacol Ther 2013;4:69–79.
89. Ocakci A, Kanter M, Cabuk M, Buyukbas S. Role of caffeic acid phenethyl ester, an active component of propolis, against NAOH-induced esophageal burns in rats. Int J Pediatr Otorhinolaryngol 2006;70:1731–9.
90. Pendeville H, Carpino N, Marine JC, Takahashi Y, Muller M, Martial JA, et al. The ornithine decarboxylase gene is essential for cell survival during early murine development. Mol Cell Biol 2001;21:6549–58.
91. Brabender J, Lord RV, Danenberg KD, Metzger R, Schneider PM, Uetake H, et al. Upregulation of ornithine decarboxylase mRNA expression in Barrett's esophagus and Barrett's-associated adenocarcinoma. J Gastrointest Surg 2001;5:174–81; discussion 82.
92. Benamouzig R, Uzzan B, Little J, Chaussade S. Low dose aspirin, COX-inhibition and chemoprevention of colorectal cancer. Curr Top Med Chem 2005;5:493–503.
93. Takashima T, Fujiwara Y, Higuchi K, Arakawa T, Yano Y, Hasuma T, et al. PPAR-gamma ligands inhibit growth of human esophageal adenocarcinoma cells through induction of apoptosis, cell cycle arrest and reduction of ornithine decarboxylase activity. Int J Oncol 2001;19: 465–71.
94. Zhao Y, Guo C, Hu H, Zheng L, Ma J, Jiang L, et al. Folate intake, serum folate levels and esophageal cancer risk: an overall and dose-response meta-analysis. Oncotarget 2017;8:10458–69.

Cancer Research
The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

AACR American Association for Cancer Research

Pathology, Chemoprevention, and Preclinical Models for Target Validation in Barrett Esophagus

Aleksandra M. Urbanska, Selvarangan Ponnazhagan and Masoud Mozafari

Cancer Res 2018;78:3747-3754. Published OnlineFirst June 29, 2018.