Published in final edited form as:

Ann N Y Acad Sci. 2011 September ; 1232: 392–400. doi:10.1111/j.1749-6632.2011.06061.x.

Barrett's esophagus and animal models

Ryan A. Macke¹, Katie S. Nason¹, Ken-ichi Mukaisho², Takanori Hattori², Takashi Fujimura³, Shozo Sasaki³, Katsunobu Oyama³, Tomoharu Miyashita³, Tetsuo Ohta³, Koichi Miwa⁴, Ali Zaidi¹, Usha Malhotra¹, Ajlan Atasoy¹, Tyler Foxwell¹, and Blair Jobe¹

¹Esophageal Cancer Program, University of Pittsburgh and University of Pittsburgh Medical Center, Hillman Cancer Center, Pittsburgh, Pennsylvania

²Department of Pathology, Shiga University of Medical Science, Shiga, Japan

³Gastroenterologic Surgery, Kanazawa University Hospital, Kanazawa, Japan

⁴Toyama Rosai Hospital, Shiga University of Medical Science, Shiga, Japan

Abstract

- Significant progress has been made in the last few decades using animal models to recreate the esophagitis–metaplasia–carcinoma sequence similar to that seen in human Barrett's esophagus (BE) and EAC. More recent works focus on molecular pathways associated with intestinal metaplasia and carcinogenesis, as well as similarities between genetic mutations occurring in humans and animal models, mouse, rat, pig, rabbit, guinea pig, dog, cat, ferret, and possum.
- Despite the lack of a perfect model, there is still significant potential in using these models to clarify the contribution of different types of reflux (gastric, biliary, and pancreatic) to esophageal adenocarcinoma and to determine how the different types of refluxate interact.
- Refluxed duodenal contents cause gastric and esophageal carcinoma in rats without exposure to carcinogens, and several rat duodenal contents reflux models have been developed. BE in the animal models has well-developed goblet cells positive for MUC2, gastric pyloric-type mucins positive for MUC6, and sometimes intermingled with gastric foveolar-type mucins positive for MUC5AC.
- A gut regenerative cell lineage, characterized by pyloric–foveolar metaplasia followed by the appearance of goblet cells, occurs in the regenerative process in response to chronic inflammation.
- High animal-fat dietary intake causes severe obesity, resulting in the development of increased abdominal pressure and increased refluxate, particularly of the duodenal

© 2011 New York Academy of Sciences.

The following on Barrett's esophagus (BE) and animal models contains commentaries on the factors of BE carcinogenesis; a duodenoesophageal reflux model; translation of targeted therapies for esophageal adenocarcinoma; and novel target regimens selected through a proteomics screen.

Conflicts of interest

The authors declare no conflicts of interest.

contents. The *N*-nitroso bile acid conjugates, which have mutagenicity, play an important role in Barrett's carcinogenesis, and are stabilized by gastric acid.

- Experiments have been made in a rodent duodeno-esophageal reflux model using thioproline or cyclooxygenase-2 inhibitor to prevent the inflammation–metaplasia–adenocarcinoma sequence. Thioproline is one of the nitrite scavengers, which reduce the production of carcinogenic nitroso-compounds. Celecoxib could postpone the sequence itself, whereas thioproline could only prevent the evolution of Barrett's esophagus to cancer.
- The Levrat's surgical model of esophago-duodenal anastomosis in rats has been shown to induce gastroduodenogastric reflux. This *in vivo* model reproduces the sequence of histologic and molecular events that lead to the development of BE and esophageal adenocarcinoma in humans and, as such, provides a realistic and translatable model for development of therapeutics for EAC.
- A pilot study using proteomics to evaluate for differentially expressed markers in the progression from metaplasia to dysplasia and ultimately adenocarcinoma in human tissues has been conducted.
- Differential expression of cytokeratin 20 in specimens from human patients and the Levrat's model substantiated the hypothesis that the animal model is representative of human cancer and, hence, further supporting the basis for its utilization.
- Furthermore, if this data is confirmed, the Levrat's approach may serve as a model for preclinical drug development. Up to ten potential novel target regimens identified and selected through the proteomics screen will be tested in a multi-arm study in rats.

Keywords

esophageal neoplasm; Barrett's esophagus; duodenogastric reflux; bile reflux; carcinoma; histogenesis; gut regenerative cell lineage; pyloric–foveolar metaplasia; fat intake; DNA adducts; *N*-nitroso bile acids; chemoprevention; duodenoesophageal reflux; thioproline; cyclooxygenase-2 inhibitor

1. Barrett's esophagus and animal models: is Barrett's carcinogenesis related to increased acid level, bile reflux, or both?

Ryan A. Macke and Katie S. Nason, nasonks@upmc.edu

It is generally agreed that BE and esophageal adenocarcinoma (EAC) develop as a result of exposure of the distal esophagus to gastroesophageal reflux. However, there is significant debate regarding which components of the refluxate are necessary and/or sufficient to produce BE/EAC. Specifically, the question of whether Barrett's carcinogenesis is related to increased acid level, bile reflux, or both remains a topic of great interest. Although acid reflux has been widely accepted as playing a key role, the necessity and sufficiency of biliary and pancreatic reflux (duodenoesophageal reflux [DER]) in BE/EAC pathogenesis has remained controversial. As with other cancers, animal models have been critically important in efforts to further elucidate the role of DER in the pathophysiology.

The ideal animal model for BE/EAC pathogenesis would possess anatomic, physiologic, and genetic similarities to humans. It has been proposed that a model with a naturally occurring squamocolumnar junction (SCJ) at the gastroesophageal junction (GEJ) and deep esophageal submucosal glands would best mimic the human esophagus. However, only a handful of animals meet these requirements and none of the models, except primates, develop BE/EAC spontaneously.

Fortunately, successful induction of EAC has been accomplished in mice, rats, and dogs (Table 1). Surgical manipulation of the foregut is the primary method used to induce epithelial metaplasia and carcinogenesis of the esophagus in animal models.

To facilitate focused analysis of the potentially harmful effects of different degrees and types of reflux (gastric, duodenal, pancreatic, and combinations thereof), a variety of foregut operations have been developed. Li *et al.* provides a useful review of some of the procedures traditionally used and their applications to guide researchers in the creation of future models¹ (Table 2).

In the majority of studies, chemical carcinogens have been used to induce esophageal cancer in surgically manipulated animals models, most notably 2,6-dimethylnitrosamine and methyl-n-amyl nitrosamine.² The results of these studies, however, have been largely contradictory and appear to be strongly influenced by the surgical manipulations used and the degree and types of reflux that are induced.³ In studies using the nitrosamine carcinogens, EAC comprised half of the tumors in rats with DER, while only ESCC was induced in those with GER.^{2,3}

In at least one study, an increasing prevalence of EAC was seen with a decreasing ratio of GER relative to DER (i.e., partial versus total gastrectomy). This finding suggests that GER may be protective in the face of DER. Interestingly, the highest prevalence of EAC in this study was in the group with only DER.³

These findings conflict with the results of other experiments, however, and further exploration is warranted. For example, Yamashita *et al.* used carcinogen with surgically created biliary, pancreatic, or pancreaticobiliary reflux in a rat model to induce esophageal carcinogenesis.⁴

GER was excluded in this model. In contrast to other studies, EAC was not induced, with all the carcinomas being ESCC. Also, biliary reflux alone did not induce a significantly higher prevalence of malignancies compared to controls, whereas pancreatic and pancreaticobiliary reflux did have a higher cancer prevalence. Finally, the necessity of an added carcinogen to induce cancer has been challenged.

Fein *et al.* successfully induced EAC in a rat model without the use of carcinogens in the late 1990s.⁵ In their study, surgical manipulation of the gastroesophageal anatomy was performed, creating DGER, DER, and no reflux groups. All rats with reflux developed esophagitis, the majority developed BE, and roughly half developed EAC by 16 weeks. There were no significant differences in the prevalence of EAC in the DGER and DER groups. Other animal models developed during this same period produced varying results,

but many of them noted that EAC could not be induced in the absence of the duodenal components of DGER.

Significant progress has been made in the last few decades using animal models to recreate the esophagitis–metaplasia–carcinoma sequence similar to that seen in human BE and EAC. Despite the lack of a perfect model, there is still significant potential in using these models to clarify the contribution of different types of reflux (gastric, biliary, and pancreatic) to EAC carcinogenesis and to determine how the different types of refluxate interact, the toxicity of the components that make up each type of refluxate, and how the acid/base environment alters the effects of these components.

More recent works are focusing on molecular pathways associated with intestinal metaplasia and carcinogenesis, as well as similarities between genetic mutations occurring in humans and animal models. This work will eventually translate into the development of diagnostic and therapeutic strategies. There is already convincing evidence that both GER and DER play significant roles in the progression to BE and EAC based on these animal models, and we anticipate that further clarity will be provided by future studies that use this valuable resource.

2. Pathogenesis and causative factors of Barrett's carcinogenesis

Ken-ichi Mukaisho and Takanori Hattori, mukaisho@belle.shiga-med.ac.jp

Pathogenesis of Barrett's esophagus and esophageal adenocarcinoma

Early lesion of Barrett's esophagus—BE develops in 5–20% of patients with chronic GERD symptoms and predisposes patients to EAC. Several epidemiological cohort studies suggest that both gastric acid and duodeno-gastroesophageal reflux induce BE, leading to increased risk of developing EAC. It has also been reported that duodenal contents reflux has great potential for malignant initiation, and refluxed duodenal contents cause gastric and esophageal carcinoma in rats without exposure to carcinogens.

To study the histogenesis of BE and EAC, several rat duodenal contents reflux models have been developed. BE leading to EAC developed in all these animal models. BE in the animal models has well-developed goblet cells positive for MUC2. Typical BE also has gastric pyloric-type mucins positive for MUC6 at their base, sometimes intermingled with gastric foveolar-type mucins positive for MUC5AC. The early lesion of BE consists of columnar cells that develop in the basal layer of the regenerative esophageal squamous epithelium are positive for MUC6 and/or MUC5AC. The mucous glands consisting of pyloric glands and foveolar cells were referred to as “pyloric–foveolar metaplasia.”⁶

Gut Regenerative Cell Lineage (GRCL) carcinogenesis—We proposed the concept of a GRCL, which occurs in the regenerative process in response to chronic inflammation.⁶ This is characterized by pyloric–foveolar metaplasia followed by the appearance of goblet cells. In addition, we found that the expression of both the intestine-specific caudal related homeobox transcription factors CDX1 and CDX2 play a crucial role in directing intestinal-type differentiation of the GRCL.⁷

The occurrence of such pyloric–foveolar metaplasia has been often reported in the gut, described as aberrant pyloric glands/pseudopyloric metaplasia in the case of Crohn’s disease, pseudopyloric gland metaplasia of the fundic stomach with chronic gastritis, gallbladder with chronic cholecystitis, gastric metaplasia in duodenal ulcers, and colonic dysplasia following ulcerative colitis, etc. We realized that there is a basic principle in regeneration of the gut. As shown in Figure 1, pyloric–foveolar metaplasia occurs at the early step, and, hence, we called this cell lineage the “GRCL.” We also suggest that GRCL is related to intestinal-type carcinogenesis.⁶

Causative factors of Barrett’s carcinogenesis

Changing patterns in the incidence of esophageal carcinoma—More than 90% of esophageal cancers are either squamous cell carcinomas (ESCC) or EAC. In 1975, approximately 75% of esophageal cancer cases diagnosed, even in the United States, were ESCC, and the remaining 25% were EAC. Among white men, the incidence of adenocarcinoma of the esophagus has increased since the mid- 1970s, surpassing that of ESCC by around 1990. It has been reported that diet is associated with GERD, and an interesting recent report showed that high dietary fat intake is associated with an increased risk of GERD symptoms and erosive esophagitis. The mechanism of this effect remains unknown, but it could at least partially explain the rising rates of GERD in the U.S. because the fat content of the food supply in the U.S. increased by 38% between 1909 and 1988.

A modified animal model—Recently, we modified a previously reported animal model and introduced ligation through a serosal suture at the esophago–jejunal junction to increase the animals’ survival rate and reduce the volume of reflux. Therefore, we successfully developed an animal model showing a higher ratio of squamous cell carcinoma compared to that of adenocarcinoma.⁸

High animal fat dietary intake as a causative factor of Barrett’s carcinogenesis

—Our hypothesis is as follows: high animal fat dietary intake causes severe obesity, resulting in the development of increased abdominal pressure and increased refluxate, particularly of the duodenal contents. Thus, this series of events raises the incidence of GERD, leading to Barrett’s carcinogenesis.

To clarify our hypothesis that high animal fat intake plays an important role in the etiology of Barrett’s carcinogenesis, the modified animal models were fed a high animal fat diet. Animals in the soybean oil group were fed the standard diet CE2 with 4.80% fat, mainly soybean oil, while animals in the high-fat group were fed a high-fat diet of Quick Fat, containing 13.9% fat, mainly beef fat. Sequential morphological changes in the animals from both groups were then studied for 30 weeks after surgery. At 30 weeks after surgery, the rats with duodenal contents reflux in the high-fat group showed a significantly higher incidence of BE and Barrett’s dysplasia than those in the soybean oil group. The incidence of EAC in the high-fat group also tended to be higher than that in the soybean oil group. We detected EAC of 16 animals in the soybean oil group, but EAC in these animals did not reach the adventitia. However, two of the six animals in the high-fat group who developed

EAC, showed expansive invasion, and one animal exhibited invasion through the adventitia into the liver.⁸

Endogenous DNA adducts and *N*-nitroso bile acids—In our previous study to elucidate the factors underlying the development of EAC, thiazolidine-4-carboxylic acid (thioprolone, TPRO) was applied to the reflux models as a nitrite scavenger.

Post-operatively, 31 animals were divided into two groups according to diet. Animals belonging to the control group were given a normal diet ($n = 18$), while the TPRO group was given food containing 0.5% TPRO ($n = 13$). All esophageal sections in both groups were histologically examined. EACs developed in 7 of 18 rats (38.9%) of the control group, whereas no EACs were detected in the TPRO group (Fisher's exact test, $P < 0.05$). Then, we suggested that nitroso compounds derived from reflux of duodenal contents play an important role in the development of EAC.⁹

In the recent study where we collaborated with National Cancer Center in Japan, Terasaki *et al.* studied the endogenous DNA adducts, which are produced from *N*-nitroso bile acid conjugates in the glandular stomach of duodenal contents reflux animals.¹⁰ The *N*-nitroso bile acid conjugates, which have mutagenicity, play an important role in Barrett's carcinogenesis.

Gastric acid as a stabilizer of *N*-nitroso bile acids—We must consider where *N*-nitroso-compounds are produced in our body. Because the esophagus and proximal cardia portion are located on the back side of the body, the esophagus is easily exposed to *N*-nitroso-compounds generated from refluxate in the stomach during sleep at night. We reported that one of the nitroso-compounds *N*-nitroso-glycocholate decomposed rapidly under alkaline conditions, but remained fairly stable under acidic condition. These findings suggest that, not only bile acids, but also gastric acid is important to retain *N*-nitroso-bile acids as a carcinogen.

Conclusions

1. The pathogenesis of BE arises from GRCL.
2. A high-fat diet plays an important role in the development of Barrett's adenocarcinoma.
3. Endogenous DNA adducts produced from *N*-nitroso bile acid conjugates play a key role in the development of Barrett's adenocarcinoma.
4. *N*-nitroso bile acids are stabilized by gastric acid.

3. Chemoprevention for esophageal adenocarcinoma using rat duodeno-esophageal reflux model

Takashi Fujimura, Shozo Sasaki, Katsunobu Oyama, Tomoharu Miyashita, Tetsuo Ohta, Koichi Miwa, and Takanori Hattori tphuji@staff.kanazawa-u.ac.jp

Introduction

A rapid increase in the incidence of esophageal adenocarcinoma (EAC) becomes a clinical problem among the Western countries. The sequence of several events progressing from gastroesophageal reflux disease to EAC is thought to involve the development of inflammation-stimulated hyperplasia and metaplasia such as Barrett's esophagus (BE), followed by multifocal dysplasia and adenocarcinoma (Fig. 2). Previously gastric juice was suggested to be an inducer of BE, dysplasia, and EAC. But recently duodenal juice was reported to be more responsible for this sequence than gastric juice.¹¹

Chemoprevention for esophageal adenocarcinoma

We have established a rodent duodeno-esophageal reflux model to induce BE and EAC.¹² Furthermore, we have reported chemopreventive effects of different chemicals such as thioproline, NSAIDs, and selective cyclooxygenase-2 inhibitors (COXIBs) on the metaplasia-dysplasia-adenocarcinoma (MDA) sequence using such models. In this paper, the outcomes of thioproline, celecoxib, and ursodeoxycholic acid (UDCA) are described.

Thiopropine

Thiopropine is one of the nitrite-trapping agents that reduce production of carcinogenic *N*-nitroso compounds. Rats receiving esophagojejunostomy, with sparing of the whole stomach, were fed with a thioproline-containing diet (TP group) or a regular diet (control group) for 45 weeks.¹³ Incidences of BE and EAC in the TP group were 67% and 17%, respectively, while they were 94% and 69% in the control group. A significant reduction in the incidence of EAC was confirmed, but there was no difference in the incidence of BE between two groups. Urinary concentration of *N*-nitrosothiopropine in the thioproline-treated group was much higher than in the control group; but in another experiment, nitrate concentration was not different between the two groups. These results strongly suggested that thioproline inhibited development of adenocarcinoma by blocking nitrosation.

Celecoxib

Cyclooxygenase-2 (COX-2) is strongly related with tumorigenesis in many cancers including esophageal adenocarcinoma.¹⁴ Celecoxib, one of the COX-2 inhibitors (COXIBs), is prescribed in the United States for patients with familial adenomatous polyposis to prevent colorectal polyps. Rats receiving esophagojejunostomy after total gastrectomy were fed with a celecoxib-containing diet (CXB group) or control diet (control group) for 40 weeks.¹⁶ Incidences of BE and EAC in the CXB group were 12% and 0%, respectively, while they were 89% and 47% in the control group (Table 3). There were significant differences between two groups in the incidence of BE and of EAC. The expression of COX-2 mRNA was strongly increased during experiments in both groups comparing to the sham operation, especially during weeks 10 to 20. However, PGE2 production of the celecoxib-treated group was greatly decreased than of the control group. Ki-67 labeling index in the control group was greatly increased compared with sham operation but significantly decreased in the celecoxib group than in the control group. On the other hand, the apoptotic index of the celecoxib group greatly increased compared to that of the control

group. These results demonstrated that celecoxib could inhibit or postpone MDA sequence by blocking COX-2 activity.

Ursodeoxycholic acid

Ursodeoxycholic acid (UDCA) is a unique hydrophilic bile acid possessing many physical functions, such as changing the proportion of bile acid, inhibiting inflammation, and reducing the NF- κ B pathway. Rats receiving esophagojejunostomy after total gastrectomy were fed a UDCA-containing diet (UDCA group) or a regular diet (control group) for 40 weeks. Incidences of BE and EAC in the UDCA group were 20% and 10%, respectively, while they were 60% and 60% in the control group. Thus, the incidences of both BE and EAC were decreased by UDCA treatment.

While the amount of UDCA usually is very small in either rodent or human bile acid, the concentration of UDCA in the UDCA-treated group was significantly higher than in the control group; this altered the proportion of bile acids in the UDCA treated group and thus the proportion of bile acids was very different between the two groups. Deoxycholic acid (DCA) and chenodeoxycholic (CDCA) acid strongly induce COX-2 through several pathways, such as NF- κ B and AP-1 pathway; UDCA stimulates these pathways less and reduces the volume of DCA and CDCA by changing bile acid proportion.

Conclusions

Duodenal reflux plays an important role in the development of esophageal adenocarcinoma. We performed chemopreventive experiments using several chemicals in a rodent duodenal reflux model. These results demonstrated that both UDCA and celecoxib could inhibit, or postpone, MDA sequence itself (DBI), whereas thioproline could prevent only development of cancer from BE by blocking nitrosation.

4. Novel approach for rapid translation of targeted therapies for esophageal adenocarcinoma

Michael K. Gibson, Ali Zaidi, Usha Malhotra, Ajlan Atasoy, Katie S. Nason, Tyler Foxwell, and Blair A. Jobe gibsonmk@upmc.edu

The incidence of esophageal adenocarcinoma (EAC) is on the rise in the United States.¹⁶ Approximately 50% of patients present with advanced disease. With this in mind, the development of improved methods and models for prevention and treatment of EAC is paramount.

Barrett's esophagus (BE), the precursor of EAC, results from chronic injury to the esophagus from acid and bile.^{17,18} The Levrat's surgical model of esophago-duodenal anastomosis in rats has been shown to induce gastroduodenal reflux.^{4,19,20} This *in vivo* model reproduces the sequence of histologic and molecular events that lead to the development of BE and EAC in humans and, as such, provides a realistic and translatable model for development of therapeutics for EAC.²¹

This translational research project aims to discover, validate, and test novel therapeutic drug targets in human EAC by:

1. using proteomic technology to discover differentially expressed proteins in BE versus EAC in human samples that represent members of aberrant pathways that may be targeted by available drugs,
2. using the Levrat's and primary heterotrans-plant animal models to screen for efficacy of these drugs against EAC, and
3. bringing the most active agent to the clinic for testing of efficacy in phase II trial for patients with advanced EAC.

Aims

1. To use proteomic technology to identify and characterize differentially expressed members of signaling pathways in human and rat BE and EAC.
 - i. **Hypothesis.** Similarly differentially expressed proteins related to the malignant phenotype of EAC in the Levrat's model and human tissue will be identified, including members of pathways for which targeted therapies are available.
 - ii. **Approach.** BE and EAC from the Levrat's model and from untreated human patients (primary surgical specimens) will be profiled by proteomics in the lab of Tom Canards. Promising targets will be evaluated for efficacy in Aim 2.
2. The primary heterotransplant (human) and Levrat's (rat) models will be used to assess the preclinical activity of agents directed against the differentially expressed protein or pathway target, as identified by proteomic profiling.
 - i. **Hypothesis.** Inhibitor(s) of differentially expressed targets will be active against rat and human EAC in these animal models.
 - ii. **Approach.** Primary heterotransplant and Levrat's models of EAC will be generated. Drugs of interest will be tested for efficacy in growth inhibition and dose response experiments.
3. To evaluate the most active agent(s) in a single-agent, phase II prospective clinical trial in patients with refractory EAC.
 - i. **Hypothesis.** Selected agents will show clinical efficacy in the trial.
 - ii. **Approach.** Prospective, single-agent, phase II clinic trial to be carried out through the UPMC Esophageal Cancer Program.

Preliminary results

We conducted a pilot study using proteomics to evaluate for differentially expressed markers in the progression from metaplasia to dysplasia and ultimately adenocarcinoma in human tissues. Differences in protein abundance from BE-, HGD-, and EAC-derived cells were determined from their spectral count values. Those significant differentially distributed

proteins were utilized for further hierarchical cluster analysis. A clear pattern was evident representing abundant level alterations of proteins that cluster with each stage of neoplastic progression.

In order to investigate shared expression of markers between human and rat tissues, we evaluated CK20 in both BE and EAC. Differential expression of this marker in specimens from human patients and the Levrat's model substantiated the hypothesis that the animal model is representative of human cancer and, hence, further support the basis for its use. We did not have paired normal tissue for both human and rat tumors, but they are being obtained. In addition, proteomic studies are ongoing.

This finding suggests that the rat model of esophageal carcinogenesis may be a representative surrogate for the same process in humans. Furthermore, if this data is confirmed, the Levrat's approach will serve as an excellent model for preclinical drug development.

Up to ten potential novel target regimens identified and selected through the proteomics screen will be tested in a multiarm study in Sprague–Dawley rats. Example agents include IGF-1R inhibitor (BMS), SMO inhibitor (BMS), SHH inhibitor (Infinity Pharmaceuticals), mTOR inhibitor (AZD and Novartis), VEGF inhibitor (Tactical Therapeutics and AZD), and an EGFR inhibitor (Amgen, BMS). Prior to testing in the animal models, dosages for each target agent shall be determined based on PK/PD work.

All drug treatment groups will be compared with a vehicle control group. During the course of the treatment, tumor volumes and weights shall be recorded routinely to determine efficacy. Active agents will be tested in human trials.

References

1. Li Y, Martin RC. Reflux injury of esophageal mucosa: experimental studies in animal models of esophagitis, Barrett's esophagus and esophageal adenocarcinoma. *Dis. esophagus*. 2007; 20:372–378. [PubMed: 17760649]
2. Attwood SE, Smyrk TC, Demeester TR, et al. Duodenoesophageal reflux and the development of esophageal adenocarcinoma in rats. *Surgery*. 1992; 111:503–510. [PubMed: 1598670]
3. Ireland AP, Peters JH, Smyrk TC, et al. Gastric juice protects against the development of esophageal adenocarcinoma in the rat. *Ann. Surg.* 1996; 224:358–370. Discussion 370–351. [PubMed: 8813264]
4. Yamashita Y, Homma K, Kako N, et al. Effect of duodenal components of the refluxate on development of esophageal neoplasia in rats. *J. Gastrointest. Surg.* 1998; 2:350–355. [PubMed: 9841992]
5. Fein M, Peters JH, Chandrasoma P, et al. Duodenoesophageal reflux induces esophageal adenocarcinoma without exogenous carcinogen. *J. Gastrointest. Surg.* 1998; 2:260–268. [PubMed: 9841983]
6. Mukaisho K, Miwa K, Hattori T, et al. Gastric carcinogenesis by duodenal reflux through gut regenerative cell lineage. *Dig. Dis. Sci.* 2003; 48:2153–2158. [PubMed: 14705821]
7. Tatsuta T, Mukaisho K, Hattori T, et al. Expression of Cdx2 in early GRCL of Barrett's esophagus induced in rats by duodenal reflux. *Dig. Dis. Sci.* 2005; 50:425–431. [PubMed: 15810620]
8. Chen KH, Mukaisho K, Hattori T, et al. High animal-fat intake changes the bile-acid composition of bile juice and enhances the development of Barrett's esophagus and esophageal adenocarcinoma in a rat duodenal-contents reflux model. *Cancer Sci.* 2007; 98:1683–1688. [PubMed: 17868414]

9. Kumagai H, Mukaisho K, Hattori T, et al. Thioproline inhibits development of esophageal adenocarcinoma induced by gastroduodenal reflux in rats. *Carcinogenesis*. 2004; 25:723–727. [PubMed: 14754873]
10. Terasaki M, Totsuka Y, Nishimura K, et al. Detection of endogenous DNA adducts, O-carboxymethyl-2'-deoxyguanosine and 3-ethanesulfonic acid-2'-deoxycytidine, in the rat stomach after duodenal reflux. *Cancer Sci*. 2008; 99:1741–1746. [PubMed: 18549406]
11. Kauer WK, Peters JH, DeMeester TR, et al. Mixed reflux of gastric and duodenal juice is more harmful to the esophagus than gastric juice alone. The need for surgical therapy re-emphasized. *Ann Surg*. 1995; 222:525–533. [PubMed: 7574932]
12. Miwa K, Segawa M, Takano Y, et al. Induction of oesophageal and forestomach carcinomas in rats by reflux of duodenal contents. *Br J Cancer*. 1994; 70:185–189. [PubMed: 8054264]
13. Sasaki S, Miwa K, Fujimura T, et al. Ingestion of thioproline suppresses rat esophageal adenocarcinogenesis caused by duodenogastroesophageal reflux. *Oncol Rep*. 2007; 18:1443–1449. [PubMed: 17982628]
14. Fujimura T, Ohta T, Oyama K, et al. Role of cyclooxygenase-2 in the carcinogenesis of gastrointestinal tract cancers: A review and report of personal experience. *World J Gastroenterol*. 2006; 12:1336–1345. [PubMed: 16552798]
15. Oyama K, Fujimura T, Ninomiya I, et al. A COX-2 inhibitor prevents the esophageal inflammation/metaplasia-adenocarcinoma sequence in rats. *Carcinogenesis*. 2005; 26:565–570. [PubMed: 15564290]
16. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2009. *CA Cancer J. Clin*. 2009; 59:225–249. [PubMed: 19474385]
17. Paulson TG, Reid BJ. Focus on Barrett's esophagus and esophageal adenocarcinoma. *Cancer Cell*. 2004; 6:11–16. [PubMed: 15261138]
18. Lagergren J, Bergstrom R, Lindgren A, et al. Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma. *N. Engl. J. Med*. 1999; 340:825–831. [PubMed: 10080844]
19. Fein M, Fuchs KH, Stopper H, et al. Duodenogastric reflux and foregut carcinogenesis: analysis of duodenal juice in a rodent model of cancer. *Carcinogenesis*. 2000; 21:2079–2084. [PubMed: 11062171]
20. Levrat M, Lambert R, Kirshbaum G. Esophagitis produced by reflux of duodenal contents in rats. *Am. J. Dig. Dis*. 1962; 7:564–573. [PubMed: 14464730]
21. Bonde P, Sui G, Dhara S, et al. Cytogenetic characterization and gene expression profiling in the rat reflux-induced esophageal tumor model. *J. Thorac. Cardiovasc. Surg*. 2007; 133:763–769. [PubMed: 17320581]

Figure 1.

GRCL carcinogenesis. The GRCL is characterized by pyloric–foveolar metaplasia with goblet cell metaplasia, which occurs in the regenerative process in response to chronic inflammation. BE very likely develops through the GRCL, and Cdx^1 and Cdx^2 play a crucial role in directing intestinal-type differentiation of the GRCL. GRCL is related to intestinal-type carcinogenesis.

Figure 2.

Anatomic and physiologic similarities of the foregut in animal models as well as pathogenesis and histopathology of esophageal cancers in these animals

Table 1

Animal Model	Does model have spontaneous ...		Anatomic features of model		Cancer develops in model?	
	Barrett's?	Reflux or vomiting?	SCJ located at anatomic GEJ?	Submucosal esophageal glands?	Physiologically induced?	Cell Type
Human	Yes	Yes	Yes	Yes	Yes	SC/AC
Mouse	No	No	No	No	Yes	SC/AC
Rat	No	No	No	No	Yes	SC/AC
Rabbit	No	No	Yes	Yes	No	N/A
Guinea Pig	No	No	Yes	Yes	No	N/A
Dog	No	Yes	Yes	Yes	Yes	AC
Cat	No	Yes	Yes	No	No	N/A
Ferret	No	Yes	Yes	Yes	No	N/A
Possum	No	No	Yes	Yes	No	N/A

SCJ, squamocolumnar junction; GEJ, gastroesophageal junction; SC, squamous carcinoma; AC, adenocarcinoma Adapted from Ref. 2.

Table 2

Summary of surgical procedures in reflux esophageal injury models

Representative surgical procedures	Esophageal injury models
Ligation of the pylorus	Esophagitis
Wendel esophagogastroplasty	Esophagitis
Total gastrectomy	Esophagitis, BE
External esophageal perfusion	Esophagitis
Mucosal excision with hiatal hernia creation	Esophagitis, BE
Esophago-jejunostomy	Esophagitis, BE, ECA
Esophago-duodenal anastomosis	Esophagitis, BE, ECA
Esophago-gastroduodenal anastomosis	Esophagitis, BE, ECA

Table 3

Post-operative weeks	Group	No. of animals examined	Incidence of	
			Barrett's esophagus	Carcinoma
10 W	Control	10	10%	0%
	Celecoxib	5	0%	0%
20 W	Control	10	40%	0%
	Celecoxib	5	20%	0%
30 W	Control	10	50%	10%
	Celecoxib	5	40%	0%
40 W	Control	19	89%	47%
	Celecoxib	8	12% ^{* *}	0% [*]

^{**}
 $p < 0.01$

^{*}
 $p < 0.05$.