Published in final edited form as:

Dis Esophagus. 2009 ; 22(4): 323–330. doi:10.1111/j.1442-2050.2008.00909.x.

Endoscopic Evaluation of Esophago-Gastro-Jejunostomy in Rat Model of Barrett's Esophagus

Shaoying Lu^{1,2}, Anson W Lowe¹, George Triadafilopoulos¹, Pei-Lin Hsiung³, Ying Hao¹, James M Crawford⁴, and Thomas D Wang^{1,5}

¹Division of Gastroenterology and Hepatology, Stanford University School of Medicine, 300 Pasteur Dr, Alway Bldg, Rm M211, Stanford, California, USA, 94305

²Department of General Surgery, First Affiliated Hospital, Medical School of Xi'an Jiaotong University, 277 West Yanta Road, Xi'an Shaanxi, People's Republic of China, 710061

³Department of Pediatrics, Stanford University School of Medicine, 300 Pasteur Dr, Alway Bldg, Rm M211, Stanford, California, USA, 94305

⁴Department of Pathology, Immunology and Laboratory Medicine, University of Florida College of Medicine, P.O. Box 100275, Gainesville, Florida, USA, 32610

⁵Division of Gastroenterology and Hepatology, University of Michigan School of Medicine, 109 Zina Pitcher Place, BSRB 1522, Ann Arbor, MI USA, 48109

Abstract

Background and aims—Endoscopy can be used to monitor the onset of metaplastic transformation and to observe the progression of neoplasia in small animal models of Barrett's esophagus. By avoiding animal sacrifice, the natural history of this disease can be studied in a longitudinal fashion. We aim to characterize the endoscopic features of esophageal mucosa at various stages of the metaplasia-dysplasia-carcinoma sequence in a rat reflux model of Barrett's for comparison with histology.

Methods—Acid and bile reflux was produced by introducing a side-to-side esophago-gastro-jejunosomy in Sprague-Dawley rats. Endoscopic examination of the distal esophagus was performed in 24 surgically-altered and 4 control rats, between weeks 24 to 36 after the operation in 4 week intervals, and all rats were biopsied and sacrificed at 36 weeks. Endoscopic images were classified based on the surface mucosal patterns of the distal esophagus and then compared to histology. The endoscopic appearance were classified as: 1) normal, characterized by a smooth surface; 2) intestinal metaplasia, defined as elevated plaques/ridges, deep grooves and thin linear folds; 3) dysplasia, indicated by coarse folds/grooves, mesh-like villi, and foveolar appearance, and 4) carcinoma, suggested by irregular shaped mass lesions with ulcerations.

Results—The endoscopic criteria for intestinal metaplasia yielded a sensitivity of 100% in comparison to histology. Intestinal metaplasia with high-grade dysplasia was found in 2 rats and with low-grade dysplasia in 3 rats. Both focally-invasive squamous cell carcinoma and invasive adenocarcinoma were found in 1 rat.

Conclusions—Small animal endoscopy in a rat model of Barrett's esophagus can be used to perform surveillance, classify mucosal patterns, observe the onset of intestinal metaplasia, and monitor the progression of neoplastic transformation, representing a useful tool for studying the natural history of this disease.

Keywords

Barrett's esophagus; rat model; endoscopy; reflux; adenocarcinoma

INTRODUCTION

Barrett's esophagus is a metaplastic transformation of the distal esophagus that results from increased exposure to chronic acid and bile reflux. Clinically, Barrett's esophagus is defined by the endoscopic appearance of "salmon pink" mucosa of variable length proximal to the gastroesophageal junction, which is confirmed histologically by the presence of intestinal metaplasia.¹ Individuals with Barrett's esophagus are at increased risk for development of esophageal adenocarcinoma,²⁻⁴ estimated at ~30 times to 125 times higher than those without esophageal intestinal metaplasia.^{5,6} The incidence of this cancer is increasing at a rate faster than any other cancer in the industrialized world.

Because the development of human Barrett's esophagus has long natural history (>10 years), small animal models are often used to study this disease. Several surgical models have been demonstrated previously to produce Barrett's esophagus in mice⁷ and rats.⁸⁻¹⁰ In the esophago-jejunostomy (EJ) model, an end-to-side anastomosis is made between the distal esophagus and the jejunum, distal to the ligament of Treitz.⁸ In the esophago-duodenostomy (ED) model, an end-to-side anastomosis is made between the distal esophagus and the duodenum distal to the pylorus.⁹ A third model entails the performance of a total gastrectomy with an end-to-side esophageal-duodenal anastomosis (TGED).¹⁰ In the last model, the incidence of adenocarcinoma is particularly high. Gene expression profiles of post-mortem esophageal specimens from these animal models demonstrate molecular homology to human disease.¹¹⁻¹³

Although endoscopy has been used to observe the development of disease in animals,^{14,15} animal sacrifice has previously been required to confirm the presence of Barrett's esophagus and determine whether adenocarcinoma is present. Hence, the natural history of this disease cannot readily be evaluated. The aim of this study is to utilize a small animal endoscope to detect the appearance of intestinal metaplasia and to monitor the progression of the metaplasia-dysplasia-adenocarcinoma sequence in a rat model. We used the esophago-gastro-jejunostomy (EGJ) model, where a side-to-side anastomosis of esophago-gastric junction is made to the jejunum distal to the ligament of Trietz,^{16,17} because it retains normal stomach function and nutritional status while allowing acid and bile to repeatedly flow back into the distal esophagus through the anastomosis.^{18,19} We reasoned that the ability of endoscopy to detect mucosal changes without sacrificing the animal can minimize the numbers of animals needed, enable accurate timing of disease onset, and offer an opportunity to use each animal as its own control. As a result, robust longitudinal studies of chemoprevention and therapeutic intervention can be performed.

MATERIALS AND METHODS

Rat Model of Esophageal Reflux

Animal experiments were conducted with approval by the Administrative Panel on Laboratory Animal Care (APLAC) at Stanford University. The EGJ procedure was performed on 32 Sprague-Dawley rats (16 male, 16 female) at ages 6 to 8 weeks. Rat chow was withheld 24 hours prior to the operation, and water was stopped on that morning. Inhaled isoflurane (3%, 0.5 ml/l volume) was used for anesthesia. After a midline laparotomy was performed, the gastroesophageal junction was identified and mobilized while carefully preserving the vagus nerve. A longitudinal incision (~1 cm) was made with

electrocautery at the esophago-gastric junction along the anti-mesenteric border of the proximal jejunum ~3 cm distal to the ligament of Treitz. While preserving the orientation and patency of the lumen, a side-to-side esophago-gastro-jejunostomy was performed using interrupted 7-0 polypropylene sutures, as shown in the schematic in Fig 1a. Normal saline (0.9% NaCl) and iron dextran (50 mg/kg) were instilled into the peritoneal cavity to compensate for blood loss. An open-and-close laparotomy sham operation was performed on 6 rats (3 male, 3 female) for use as controls.

Topical bacitracin ointment was then applied to the post-operative wound site to prevent infection. Water with acetaminophen (1 mg/ml) was provided to the rats for post-anesthesia pain control, and the animals were fed on the first post-operative day. The rats were housed 2 per cage under standard laboratory conditions ($20\pm 2^{\circ}\text{C}$, $50\pm 10\%$ relative humidity, and 12 hour light/dark cycles). All rats were sacrificed at 36 weeks after the operation. In the EGJ configuration, acid and bile reflux into the distal esophagus through the anastomosis where the lower esophageal sphincter has been weakened. The development of intestinal metaplasia and carcinoma is shown in the autopsy specimen in Fig 1b.

Small Animal Endoscopy

The small animal endoscope consists of a rigid Hopkins II, 0 degree, straight telescope with a 9.5 Fr (3 mm) outer diameter, 11.5 cm working length, and 3 Fr (1 mm) instrument channel (Karl Storz Veterinary Endoscopy, Goleta, CA), as shown in Fig 2a. A 175 W xenon lamp delivered illumination light through a flexible light guide. White light reflectance images were detected with a 3-chip color camera that has an integrated parfocal zoom lens (Tricam PDD) with NTSC (National Television System Committee) output. A pinch forceps with a cup size of ~1mm and double action jaws passed through the midline instrument port of the endoscope to collect tissue specimens, as shown in Fig 2b. Endoscopy was performed after fasting the rats overnight and administering isoflurane, as described above. The animals were placed in the supine position, and the extremities were restrained to prevent injury. The endoscope was advanced into the distal esophagus by direct visualization using ~1 to 2 ml of air injected through a side instrument port to dilate the lumen. Acetic acid (0.1 ml at 3%) was topically applied to the distal esophagus for ~2 minutes followed by rinsing with water to enhance contrast, and the mucosal surface was then carefully examined. A complete endoscopy evaluation was performed in < 3 minutes. Biopsies were performed in all of the rats prior to sacrifice at 36 weeks. Control rats were studied in the same way, but without biopsy. All images obtained during endoscopy were recorded using a digital video recorder (Sony DVD-1000MD, Sony Electronics Inc, Tokyo, Japan).

Histological examination

Endoscopic biopsies were embedded in OCT freezing compound, cut in 6 μm sections, fixed in acetone at 4°C for 90 seconds, and stained with hematoxylin and eosin (H&E). After animal sacrifice at 36 weeks, the entire esophagus, including the anastomosis and a 5 mm length of jejunum, was removed and divided longitudinally (Fig 1b). The specimen was spread onto a cork plate with mucosal side facing up for gross macroscopic examination, and then fixed in 10% buffered formalin. Complete fixation was achieved after 48 hours, and the resected tissues were then cut serially into 3 mm slices along the longitudinal axis of the esophagus. These segments were embedded in paraffin, cut into 4 μm sections, and stained with H&E. All histologic specimens were examined in a blinded fashion by an experienced gastrointestinal pathologist (JMC). According to *a priori* definitions, the specimens were classified histologically as follows: 1) esophageal squamous mucosa with mild, moderate, or severe basal zone hyperplasia; 2) intestinal metaplasia (Barrett's esophagus) with no, mild, or moderate hyperplasia, but no dysplasia; 3) intestinal metaplasia

(Barrett's esophagus) with low or high-grade dysplasia; 4) gastric cardia/fundic mucosa with no, minimal, or moderate foveolar cell hyperplasia; 5) adenocarcinoma; and 6) squamous cell carcinoma.^{22,23} Specimens with insufficient tissue for evaluation were excluded. In the autopsy examinations, histological classification of both the squamous mucosa, and the region of Barrett's esophagus, was of the most severe disease observed.

Data analysis

The weights of the rats were recorded to assess the effect of the operation on nutritional status. The experimental and control groups were weighed prior to the surgical procedure (age 6 to 8 weeks) and every 2 weeks thereafter, and the results were normalized to the pre-operation value. Endoscopic observations were made between 24 to 36 weeks post-operatively in 4 week intervals. The mucosal surface of the distal esophagus was classified endoscopically according to the following patterns: 1) normal appearance, characterized by smooth surface and distinct vascular pattern, representing squamous mucosa; 2) elevated plaques/ridges, deep grooves, thin linear folds, and reduced vascular pattern, suggesting esophagitis or intestinal metaplasia; 3) coarse folds/grooves, mesh-like villous, and foveolar appearance and hyperemic vascular pattern, signifying dysplasia; 4) irregular shaped mass lesions with ulcerations and uneven, rough, friable mucosa, indicating carcinoma. The mucosal surface of the distal esophagus was also classified post-mortem by gross observation according to extent of luminal dilation, presence of folds, nodules, and ulcers. Finally, histological evaluation of biopsy specimens acquired through the endoscope at 36 weeks and at autopsy was performed and compared to the endoscopy results.

RESULTS

Animal Survival

The normalized weight of the rats ranged from 183 to 370 gm and from 222 to 392 gm over the course of the study for the experimental and controls group, respectively. The reflux model rats had an average normalized weight that was slightly less than that of the control rats for the first 8 weeks following the surgery, and then become approximately the same thereafter, as shown in Fig. 3. This result demonstrates adequate nutritional status for the surgically-altered rats, an important feature of the esophago-gastro-jejunostomy procedure.

The overall mortality rate in the experimental rats was 25% (8/32). A total of 4 surgically-altered rats died within 48 hours after operation because of bleeding, 3 rats died from jejunal obstruction before endoscopy could be performed, and 1 rat died of unknown reasons. Non-fatal luminal intra-operative bleeding was observed in 3 surgically-altered rats, that stopped spontaneously within a few minutes. The control rats were initially evaluated by endoscopy, and 2 of them died ~10 min after the procedure from aspiration. Subsequent endoscopy was performed by first removing the gastric intra-luminal contents with suction applied by a syringe to the midline instrument port, and no further endoscopy-related deaths occurred. In total, 24 surgically-altered rats and 6 control rats were evaluated by endoscopy and biopsy.

Endoscopic observations

The endoscopic observations from the 24 surgically-altered and 6 control rats are summarized in Table 1. Note that some of the animals had multiple mucosal findings at different locations in the latter stages of disease development. The endoscopic appearance of Barrett's esophagus was observed in 58.3% rats (14/24) and was identified from the appearance of any of the following mucosa patterns: thin linear (3/24), deep linear (7/24), villous (2/24) and foveolar (2/24) in the 36 weeks post-operational endoscopic examination. In three rats multi-focal deep linear patterns were found intermittently along the esophagus. None of the 24 rats at 36 weeks displayed salmon-colored mucosa characteristic of intestinal

metaplasia, as found in human esophagus. Representative endoscopic findings are shown in Fig 4. Endoscopic examination of the 4 long-term surviving control rats did not reveal any abnormal findings throughout the course of the study (24 to 36 weeks). In addition, jejunal mucosa appeared smooth and pink and could be easily distinguished from intestinal metaplasia endoscopically.

Gross post-mortem observations

At autopsy, in 79% (19/24) of the surgically-altered rats the esophagus lumen appeared dilated and the distal one-third to one-half of the esophageal mucosa was observed to be grossly abnormal. The inner surface of esophagus showed irregular longitudinal folds and whitish nodular patches, resembling a cobblestone appearance. In addition to these changes, focal and extensive ulcerations were found in 29% (7/24) rats. In one of these animals, a tumor was detected and confirmed on histology to be adenocarcinoma (Fig. 1b). The tumor was surrounded by gross changes that included linear folds with elevated plaques/ridges and deep grooves, found on histology to be intestinal metaplasia. In 21% (5/24) of the surgically-altered rats, the esophageal lumen was not dilated and the remaining excised specimen appeared grossly normal.

Histological observations on biopsy specimens

Biopsy specimens acquired through the endoscope at 36 weeks were evaluated in the experimental and control rats. Histological findings in the surgically-altered rats (n=24) include squamous epithelium (n=16) and intestinal metaplasia (n=8), summarized in Table 2. Both specimens of squamous epithelium with basal zone hyperplasia were scored as mild, and one specimen of dysplasia was found. There were no findings of squamous cell carcinoma or adenocarcinoma on these specimens. Representative images of histology from a) squamous epithelium, b) intestinal metaplasia, c) dysplasia, and d) basal zone hyperplasia are shown in Fig 5. Only squamous mucosa was found on the specimens obtained from the control rats.

Histological observations on post-mortem specimens

The final tissue diagnoses were made from post-mortem specimens, summarized in Table 2. The histological features representative of a) squamous epithelium, b) intestinal metaplasia, c) dysplasia, and d) adenocarcinoma are shown in Fig 6. Autopsy documented squamous mucosa in 100% of the surgically-altered rats (24/24). Basal zone hyperplasia was found primarily in the distal one-third of the esophagus in 50% of these rats (12/24). The salient feature of this finding was reactive hyperplasia, and was scored as mild, moderate, or severe. Inflammation with neutrophils or eosinophils was not observed. Additional histologic findings in the squamous mucosa included infiltration with inflammatory cells, hyperkeratinization, extensive epithelial sloughing, and ulceration. Intestinal metaplasia was found in 8 of the surgically-altered rats (33%), and was characterized by the replacement of the stratified squamous mucosa with a glandular columnar mucosa. In one rat, both focally invasive adenocarcinoma and squamous cell carcinoma were found in adjacent areas without the presence of dysplasia.^{20,21} In the surgically-altered rats that had the endoscopic appearance of Barrett's esophagus, histology revealed 2 with high grade and 3 with low grade dysplasia.

Comparison of endoscopic and histological findings

We combined the histological findings from endoscopy at 36 weeks after operation and post-mortem specimens for comparison with the endoscopic evaluation. The elevated plaque/ridge pattern was associated with mild hyperkeratosis, epithelial proliferative activity with acanthosis, and epithelial projection. The formation of irregular elevated regions of the

mucosa was associated with dysplasia and/or invasive squamous cell carcinoma and adenocarcinoma. Barrett's epithelium associated with thin linear folds (1/24), deep linear folds (3/24), mesh-like villous (2/24), and foveolar hyperplasia (2/24) on endoscopy was confirmed on histology. In 2 rats, coarse linear folds were found endoscopically while the diagnosis was not confirmed by histological findings. Overall, the endoscopic findings of intestinal metaplasia were highly consistent with the histological results. Compared with histology, the diagnosis of intestinal metaplasia on endoscopy yielded a sensitivity of 100% (8/8) and a specificity of 50% (8/16) at 36 weeks after the operation. The histological features found at autopsy in the distal esophageal specimens from the 24 surgically-altered rats are summarized in Table 2.

DISCUSSION

Here we demonstrate the use of a small animal endoscope to monitor the onset of intestinal metaplasia and the development of neoplasia in an esophago-gastro-jejunostomy rat model. We also classify the endoscopic mucosal appearance of the distal esophagus over a period of 24 to 36 weeks. Moreover, this approach has been used to devise classification schemes to identify mucosal patterns that correlate with the presence of neoplastic changes.^{22,23} Our findings are consistent with the criteria established by clinical endoscopic studies for identifying squamous epithelium, intestinal metaplasia, dysplasia, and carcinoma.^{24,25}

The endoscopic findings are consistent with the histology results from biopsy and autopsy, suggesting that endoscopy is useful for the detecting and observing the development of intestinal metaplasia over time. This approach can be used to monitor the time course of biomarker expression in the esophagus that results from chronic exposure to acid and bile.^{26,27} Moreover, endoscopic imaging in animal models may help provide a better understanding of the etiology, cellular origin and pathogenesis of intestinal metaplasia and adenocarcinoma. This technique may also help discover new cancer biomarkers, develop molecular probes, and devise treatment strategies.

In previous studies, sequential endoscopic examinations of esophageal carcinogenesis have been induced by carcinogens, such as N-nitrosomethyl-benzylamine (NMBA).^{28,29} Redness of the mucosa, focal loss of the visible blood vessel network, plaque-like elevated lesions, and smooth and irregular polyps were found. Each endoscopic finding was correlated with histological changes, suggested that longitudinal follow-up with endoscopy may be useful for monitoring the carcinogenesis process. Elevated plaques/ridges were also observed on endoscopy, and were found on histology to be associated with chronic esophagitis, characterized by mild hyperkeratosis and epithelial proliferation. Also, irregular polyps found on endoscopy correlated with esophagitis and squamous cell carcinoma on histology. In this study, the specificity of endoscopic diagnosis of intestinal metaplasia decreased from 67% (week 24) to 50% (weeks 32 and 36), as endoscopic visualization became limited by the development of ulceration and inflammation.

The reflux model used in this study has a number of advantages for studying the development of Barrett's esophagus and esophageal adenocarcinoma in small animals, including 1) the EGJ procedure is easy to perform; 2) reflux of both acid and bile occurs; and 3) gastric function, weight and nutritional status are preserved. These features are reflected by the high rate of animal survival, and the ability of the surgically-altered rats to achieve rapid gain in body weight and eventually catch up to that of the control rats. In the other rat models, the stomach is bypassed, and the animals suffer post-operatively from iron-deficiency anemia, inability to gain weight, and decreased survival. The percent of rats that developed adenocarcinoma was less than that found in other studies because all animals were

sacrificed at 36 weeks to minimize suffering from obstruction that might have occurred with onset of malignancy.

Longitudinal evaluation of this animal model using endoscopy can provide insights into the development of Barrett's epithelium. Prevailing theories regarding the origin of Barrett's neoplasia include: 1) glandular epithelium from the gastro-esophageal junction creeps into and replaces defective esophageal squamous mucosa,³⁰ or 2) intestinal metaplasia originates *de novo* from squamous stem cells in the esophagus.³¹ Our results from this study support the latter hypothesis, as endoscopic findings of deep linear, mesh-like villous, and foveolar mucosa patterns were found primarily at the junction of normal esophagus and mucosa associated with esophagitis, which is remote from the site of anastomosis. These observations corresponded to sporadic and multi-focal intestinal metaplasia on histology.

Moreover, low and high-grade dysplasia was found in 3 rats adjacent to regions of intestinal metaplasia, and 1 rat had intestinal metaplasia and invasive adenocarcinoma next to high-grade squamous dysplasia and focally invasive squamous cell carcinoma. Other studies have also found both adenocarcinoma and squamous cell carcinomas in the mucosa with intestinal metaplasia.¹⁶ The occurrence of a mixture of columnar and squamous cells in the same or adjacent regions of mucosa support the above-mentioned hypothesis that proliferative cells appear with the potential to differentiate into either columnar or squamous morphology. In the future, longitudinal evaluation of this animal model with endoscopy promises to provide further insights into mechanisms for development of Barrett's epithelium and its transformation to neoplasia.

Acknowledgments

The authors acknowledge funding support from the National Institutes of Health, including P30 DK56339 (SL, TDW), U54 CA105296 (JC, TDW), R01 DK063624 (GT, AWL, YH), R33 CA109988 (TDW), and K08 DK067618 (TDW). PH acknowledges support from the American Cancer Society Canary Foundation Postdoctoral Fellowship.

REFERENCES

1. Spechler SJ. Barrett's Esophagus. *N Engl J Med*. 2002; 346:836–842. [PubMed: 11893796]
2. Hornick JL, Odze RD. Neoplastic precursor lesions in Barrett's esophagus. *Gastroenterol Clin North Am*. 2007; 36:775–796. [PubMed: 17996790]
3. Mueller J, Werner M, Stolte M. Barrett's esophagus: histopathologic definitions and diagnostic criteria. *World J Surg*. 2004; 28:148–154. [PubMed: 14727064]
4. Jankowski JA, Harrison RF, Perry I, Balkwill F, Tselepis C. Barrett's metaplasia. *Lancet*. 2000; 356:2079–2085. [PubMed: 11145505]
5. 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; 53:1070–1074. [PubMed: 15247170]
6. American Cancer Society. *Cancer Facts & Figures 2008*. Atlanta: American Cancer Society; 2008. p. 1
7. Xu X, LoCicero J 3rd, Macri E, Loda M, Ellis FH Jr. Barrett's esophagus and associated adenocarcinoma in a mouse surgical model. *J Surg Res*. 2000; 88:120–124. [PubMed: 10644476]
8. Buttar NS, Wang KK, Leontovich O, Westcott JY, Pacifico RJ, Anderson MA, Krishnadath KK, Lutzke LS, Burgart LJ. Chemoprevention of esophageal adenocarcinoma by COX-2 inhibitors in an animal model of Barrett's esophagus. *Gastroenterology*. 2002; 122:1101–1112. [PubMed: 11910360]
9. Goldstein SR, Yang GY, Curtis SK, Reuhl KR, Liu BC, Mirvish SS, Newmark HL, Yang CS. Development of esophageal metaplasia and adenocarcinoma in a rat surgical model without the use of a carcinogen. *Carcinogenesis*. 1997; 18:2265–2270. [PubMed: 9395230]

10. Ireland AP, Peters JH, Smyrk TC, DeMeester TR, Clark GW, Mirvish SS, Adrian TE. Gastric juice protects against the development of esophageal adenocarcinoma in the rat. *Ann Surg.* 1996; 224:358–370. [PubMed: 8813264]
11. Greenawalt DM, Duong C, Smyth GK, Ciavarella ML, Thompson NJ, Tiang T, Murray WK, Thomas RJ, Phillips WA. Gene expression profiling of esophageal cancer: comparative analysis of Barrett's esophagus, adenocarcinoma, and squamous cell carcinoma. *Int J Cancer.* 2007; 120(9): 1914–1921. [PubMed: 17236199]
12. Cheng P, Gong J, Wang T, Chen J, Liu GS, Zhang R. Gene expression in rats with Barrett's esophagus and esophageal adenocarcinoma induced by gastroduodenoesophageal reflux. *World J Gastroenterol.* 2005; 11(33):5117–5122. [PubMed: 16127739]
13. Hao Y, Triadafilopoulos G, Sahbaie P, Young HS, Omary MB, Lowe AW. Gene expression profiling reveals stromal genes expressed in common between Barrett's esophagus and adenocarcinoma. *Gastroenterology.* 2006; 131(3):925–933. [PubMed: 16952561]
14. Taylor P, Armstrong D, Linsell J, Power S, Mason R. Sequential endoscopy and biopsy of intact rat stomach. A new simple technique. *Dig Dis Sci.* 1988; 33:321–323. [PubMed: 3342724]
15. Meyrat P, Baumgartner A, Kappeler M, Halter F. Simple gastroscopy technique in the rat. *Dig Dis Sci.* 1984; 29:327–329. [PubMed: 6705645]
16. Kumagai H, Mukaisho K, Sugihara H, Bamba M, Miyashita T, Miwa K, Hattori T. Cell kinetic study on histogenesis of Barrett's esophagus using rat reflux model. *Scand J Gastroenterol.* 2003; 38:687–692. [PubMed: 12889552]
17. Kumagai H, Mukaisho K, Sugihara H, Miwa K, Yamamoto G, Hattori T. Thioproline inhibits development of esophageal adenocarcinoma induced by gastroduodenal reflux in rats. *Carcinogenesis.* 2004; 25(5):723–727. [PubMed: 14754873]
18. Pera M, Brito MJ, Poulson R, Riera E, Grande L, Hanby A, Wright NA. Duodenal-content reflux esophagitis induces the development of glandular metaplasia and adenosquamous carcinoma in rats. *Carcinogenesis.* 2000; 21:1587–1591. [PubMed: 10910963]
19. Clark GW, Smyrk TC, Mirvish SS, Anselmino M, Yamashita Y, Hinder RA, DeMeester TR, Birt DF. Effect of gastroduodenal juice and dietary fat on the development of Barrett's esophagus and esophageal neoplasia: an experimental rat model. *Ann Surg Oncol.* 1994; 1:252–261. [PubMed: 7842295]
20. Buskens CJ, Hulscher JB, van Gulik TM, Ten Kate FJ, van Lanschot JJ. Histopathologic evaluation of an animal model for Barrett's esophagus and adenocarcinoma of the distal esophagus. *J Surg Res.* 2006; 135:337–344. [PubMed: 16926029]
21. Su Y, Chen X, Klein M, Fang M, Wang S, Yang CS, Goyal RK. Phenotype of columnar-lined esophagus in rats with esophagogastrroduodenal anastomosis: similarity to human Barrett's esophagus. *Lab Invest.* 2004; 84:753–765. [PubMed: 15094711]
22. Rey JF, Inoue H, Guelrud M. Magnification endoscopy with acetic acid for Barrett's esophagus. *Endoscopy.* 2005; 37:583–586. [PubMed: 15933935]
23. Guelrud M, Ehrlich EE. Endoscopic classification of Barrett's esophagus. *Gastrointest Endosc.* 2004; 59:58–65. [PubMed: 14722549]
24. Mayinger B, Oezturk Y, Stolte M, Faller G, Benninger J, Schwab D, Maiss J, Hahn EG, Muehldorfer S. Evaluation of sensitivity and inter- and intra-observer variability in the detection of intestinal metaplasia and dysplasia in Barrett's esophagus with enhanced magnification endoscopy. *Scand J Gastroenterol.* 2006; 41:349–356. [PubMed: 16497625]
25. Fortun PJ, Anagnostopoulos GK, Kaye P, James M, Foley S, Samuel S, Shonde A, Badreldin R, Campbell E, Hawkey CJ, Ragnath K. Acetic acid-enhanced magnification endoscopy in the diagnosis of specialized intestinal metaplasia, dysplasia and early cancer in Barrett's oesophagus. *Aliment Pharmacol Ther.* 2006; 23:735–742. [PubMed: 16556175]
26. Melo LL, Krul CD, Kliemann LM, Cavazzola LT, Boeno Rda L, Silber PC, Grossi RS. Influence of surgically induced gastric and gastroduodenal content reflux on esophageal carcinogenesis - experimental model in Wistar female rats. *Dis Esophagus.* 1999; 12:106–115. [PubMed: 10466042]

27. Nishijima K, Miwa K, Miyashita T, Kinami S, Ninomiya I, Fushida S, Fujimura T, Hattori T. Impact of the biliary diversion procedure on carcinogenesis in Barrett's esophagus surgically induced by duodeno-esophageal reflux in rats. *Ann Surg.* 2004; 240:57–67. [PubMed: 15213619]
28. Baden T, Yamamichi K, Michiura T, Tsubura A, Hioki K. Sequential endoscopic findings and histological changes of N-nitrosomethylbenzylamine-induced esophageal carcinogenesis in rats. *Oncol Rep.* 2006; 16:965–970. [PubMed: 17016578]
29. Reen RK, Dombkowski AA, Kresty LA, Cukovic D, Mele JM, Salagrama S, Nines R, Stoner GD. Effects of phenylethyl isothiocyanate on early molecular events in N-nitrosomethylbenzylamine-induced cytotoxicity in rat esophagus. *Cancer Res.* 2007; 67:6484–6492. [PubMed: 17616710]
30. 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(1):209–216. [PubMed: 10483471]
31. Li H, Walsh TN, O'Dowd G, Gillen P, Byrne PJ, Hennessy TP. Mechanisms of columnar metaplasia and squamous regeneration in experimental Barrett's esophagus. *Surgery.* 1994; 115:176–181. [PubMed: 8310406]

Fig. 1. Rat Esophago-Gastro-Jejunostomy (EGJ) Model of Barrett's Esophagus

a) A side-to-side anastomosis of the distal jejunum and esophago-gastric junction causes acid and bile to reflux into the distal esophagus. **b)** Intestinal metaplasia appears in the distal esophagus ~24 weeks afterwards as irregular linear folds with white plaques/ridges and deep grooves. The irregularly shaped mass marked by the group of three black arrows was found to be adenocarcinoma.

Fig. 2. Small animal endoscope

a) A rigid endoscope is used to perform imaging, and has instrument ports for insufflating the lumen of the esophagus. White light provided by a xenon lamp is delivered through the light guide, and the images are detected with a color camera. **b)** Forceps are passed through the midline instrument port to collect tissue biopsies.

Fig. 3. Weight gain in reflux model rats

The average weight of the surgically-altered rats (normalized to that taken prior to the operation) is initially less than that of the control group but becomes approximately equivalent after 8 weeks. This result demonstrates an important feature of the EGJ model for preserving gastric function, error bars represent standard deviation.

Fig. 4. Endoscopic images of distal esophagus

a) squamous epithelium from normal rat esophagus has a smooth surface and distinct vascular pattern; **b)** intestinal metaplasia appears as elevated plaques/ridges, deep grooves, or thin linear folds with a reduced vascular pattern; **c)** dysplasia presents as coarse folds/grooves, mesh-like villous, or foveolar appearance with a hyperemic vascular pattern, **d)** adenocarcinoma manifests as irregular shaped mass lesions with ulcerations and uneven, rough, friable mucosa.

Fig. 5. Biopsy specimens of distal esophagus

Representative histological findings on biopsy include: **a)** squamous epithelium, **b)** intestinal metaplasia, **c)** dysplasia, and **d)** basal zone hyperplasia; scale bar 50 μm .

Fig. 6. Autopsy specimens of distal esophagus

Representative histological findings on full thickness sections from autopsy include: **a)** squamous epithelium, **b)** intestinal metaplasia, **c)** dysplasia, and **d)** adenocarcinoma, scale bar 50 μm .

Summary of endoscopic findings in surgically-altered rats (n = 24) over period of 24 to 36 weeks. Multiple endoscopic findings were observed at different regions of the mucosa in some rats at latter time points.

Table 1

Endoscopic Description	Vascular Pattern	Associated Histology	Weeks after operation			
			24	28	32	36
normal appearance, smooth surface	distinct	squamous	8	5	5	5
elevated plaques/ridges, deep grooves, linear folds	reduced	metaplasia	12	13	16	16
coarse folds/grooves, mesh-like villous, foveolar	hyperemic	dysplasia	2	2	4	4
irregular shaped mass lesions, ulcerations	friable	carcinoma/esophagitis	2	4	6	6

Table 2

Summary of histologic findings in surgically-altered rats (n = 24) on biopsy and at autopsy. Multiple findings are found in some specimens.

Histological Evaluation	Biopsy	Autopsy
squamous epithelium	16	24
basal zone hyperplasia	2	12
mild	2	3
moderate	0	6
severe	0	3
intestinal metaplasia (Barrett's esophagus)	8	8
intestinal metaplasia with dysplasia	1	3
adenocarcinoma	0	1
squamous cell carcinoma	0	1