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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Columnar Metaplasia in Three Types of Surgical Mouse Models of Esophageal Reflux

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SUMMARY

The esophagogastrojejunostomy model, esophagogastric junction, and jejunum side-to-side anastomosis, which causes reflux of gastric acid and duodenal content, developed columnar metaplasia and dysplasia most frequently in mice, compared with esophagojejunostomy (end-to-side) with esophagogastric separation (esophagojejunostomy) and esophagojejunostomy (end-to-side) with total gastrectomy. The mortality rate of the esophagogastrojejunostomy model was 13.0%. Columnar metaplasia developed in 45.5% of mice and dysplastic columnar metaplasia developed in 21.2% of mice.

BACKGROUND AND AIMS: Esophageal adenocarcinoma develops in the setting of gastroesophageal reflux and columnar metaplasia in distal esophagus. Columnar metaplasia arising in gastroesophageal reflux models has developed in rat; however, gastroesophageal reflux models in mice have not been well-characterized.

METHODS: One hundred thirty-five C57Bl/6J mice aged 8 weeks old were divided into the following operations: esophagogastrojejunostomy (side-to-side) (EGJ), esophageal separation and esophagojejunostomy (end-to-side) (EJ), and EJ and gastrectomy (end-to-side) (EJ/TG). The animals were euthanized after 40 weeks and the histology of the junction was examined. Immunohistochemistry for p53, PDX-1, and CDX-2 was performed.

RESULTS: Metaplasia developed in 15/33 (45.5%) of EGJ, 0/38 (0%) of EJ, and 6/39 (15.4%) of EJ/TG ($P < .05$) and dysplasia developed 7/33 (21.2%) of EGJ, 0% of EJ, and 1/39 (2.6%) of EJ/TG. p53 was positive in all of the dysplastic regions, 12/15 (80%) metaplasias in the EGJ model, and 1/6 (16.7%) metaplasia in the EJ/TG model. CDX-2 was positive in all cases of metaplasias, but decreased in some cases of dysplasia. PDX-1 was positive in 7/8 (88%) cases of dysplasia and in 15/21 (71%) cases of metaplasia ($P < .05$).

CONCLUSIONS: The EGJ model, which causes reflux of gastric acid and duodenal content, developed metaplasia and dysplasia most frequently. No metaplasia developed in the EJ model in which gastric juice and duodenal content mixed before reflux. Thus, duodenal contents alone can induce columnar metaplasia and dysplasia; however, the combination of gastric acid with duodenal content reflux can cause metaplasia and dysplasia more efficiently. *(Cell Mol Gastroenterol Hepatol 2017;4:115–123; http://dx.doi.org/10.1016/j.jcmgh.2017.03.009)*

Keywords: GERD; Esophageal Reflux; Barrett's Esophagus; Esophageal Adenocarcinoma.

See editorial on page 183.

The incidence of adenocarcinoma of the esophagus is increasing in Western countries.[1,2] The reasons for this increase are not clear, and the most cited risk factors for this neoplasia are obesity and gastroesophageal reflux disease (GERD).[3] It is believed that GERD stimulates the progression from normal stratified epithelium to columnar epithelium (intestinal metaplasia, or Barrett's esophagus) and from this columnar epithelium to esophageal adenocarcinoma. Given that GERD is a common diagnostic finding[4] but that only a small fraction of these patients develop adenocarcinoma,[5] important factors in the process are still unknown.

Some animal surgical models have been used to study this process, mainly with rats.[6,7] Surgical GERD models with rats are good models for pathologic analysis and are easy to handle because of animal size. However, the availability of genetic modified strains is much superior for mouse, which encouraged a few authors to try experimental mouse models.[8] We also developed a mouse GERD model; however, the rate of occurrence of metaplasia was 45%, lower than in rat models.[9] In this report, we compare 3 surgical mouse models of esophageal reflux, including our former model, to evaluate which model is best for studying GERD.

Homeobox genes play important roles in the development of gastrointestinal tract and specific homeobox genes are expressed in normal gastrointestinal mucosa with head-tail axis. CDX-2 is a homeobox gene expressed in intestinal

Abbreviations used in this paper: **AB, alcian blue; EGJ, esophagogastrojejunostomy; EJ, esophagojejunostomy; GERD, gastroesophageal reflux disease; PAS, periodic acid–Schiff; TG, gastrectomy.**

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development[9] that has been shown to be central to the formation of intestinal metaplasia and Barrett's esophagus.[10,11] Another homeobox gene that has been implicated in the genesis of intestinal metaplasia is PDX-1, which has a role in the formation of the gastric antrum, duodenum, and pancreas. In our former report, all the human intestinal metaplasia of stomach was PDX-1 positive, and we concluded that intestinal metaplasia in the stomach is duodenal metaplasia.[12] Here, we compare the expressions of these homeobox genes in columnar metaplasia induced by the 3 models in mice and confirm that the columnar metaplasia in mouse models displays aspects similar to those seen in human Barrett's epithelium.[13]

Materials and Methods

C57Bl/6J male mice aged 8 weeks were purchase from Charles River Laboratories Japan (Yokohama, Japan), housed according to accepted standards,[14] and had free access to regular food (CMF, Oriental Yeast Co, Chiba, Japan) and water. One hundred forty-four mice were divided into 4 groups, 9 mice for a sham-operated control group and 3 types of operations (Figure 1): (1) 46 mice for side-to-side esophagogastrojejunostomy (EGJ), (2) 43 mice for esophageal separation and esophagojejunostomy (EJ), and (3) 46 mice for gastrectomy (TG) and EJ. We performed all operations under general anesthesia; the mice were fasted from the night before until the morning after the procedure, with no restriction of water intake. When appropriate, ligation of the esophagogastric junction and the gastroduodenal segment was done with 4–0 silk; the anastomoses were performed in an interrupted fashion, with 8–0 silk. After the procedure, the animals were followed for 40 weeks with weight measuring and were euthanized using pentobarbital. This study protocol was conducted in accordance with the ARRIVE guidelines and was approved by the animal ethics committee of the University of Tokyo.

The specimens were prepared with a combination of intravenous perfusion and immersion of 4% formaldehyde followed by immersion in alcohol 70%. Paraffin blocks were prepared and serial 5-μm sections were cut. These were processed by hematoxylin-eosin staining for histologic assessment and by the periodic acid–Schiff/alcian blue (pH 2.5) (PAS/AB) method for mucin staining. For the immunohistochemical analyses, antigen retrieval was performed with microwave (H2800, Energy Beam Sciences, Agawam, MA) or autoclave (2100 Retriever, Prestige Medical, Lelystad, The Netherlands) using as buffer solutions sodium citrate (pH 6) or Tris-EDTA (pH 9). Primary antibodies used were the proliferative marker Ki-67 (rat, 1:50, Dako, Tokyo, Japan), CDX-2 (mouse, 1:80, Biogenex, San Ramon, CA), p53 (rabbit, 1:1000, Novocastra, Vista, CA), PDX-1 (rabbit polyclonal, 1:5000, a

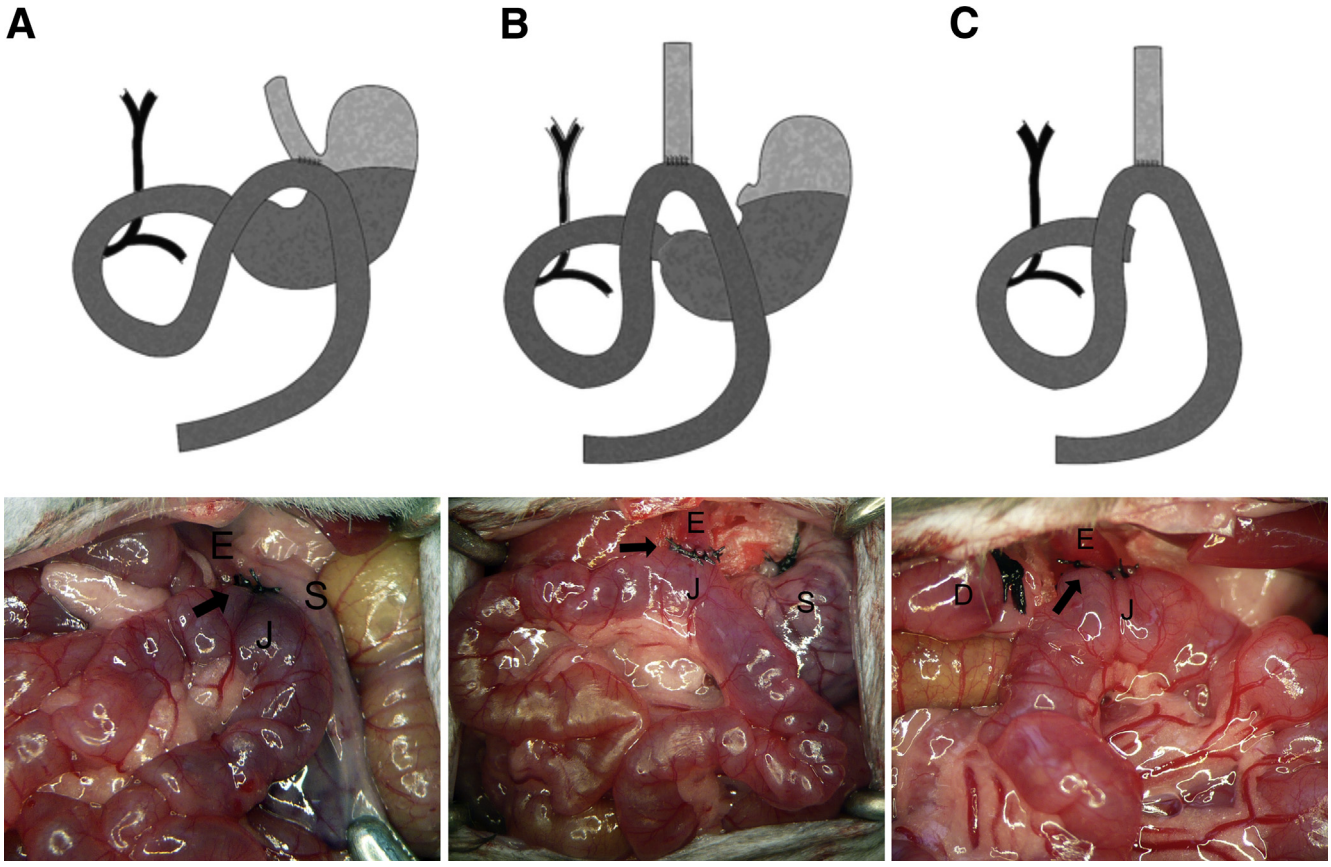

Figure 1. Types of operations. (*A*) EGJ. (*B*) Esophageal separation and EJ. (*C*) EJ/TG. EGJ has reflux of gastric content and intestinal content periodically. EJ has reflux of mixture of gastric content and intestinal content. EJ/TG has reflux of intestinal content without gastric acid. D, duodenum; E, esophagus; J, jejunum; S, stomach. Scale bar: 5 mm. *Arrows*, anastomosis.

kind gift from Chris Wright, Vanderbilt University), TFF-1 (rabbit, 1:5000, a kind gift from Yasukazu Ohmoto), and TFF-2 (mouse, 1:80, a kind gift of Nicholas Wright and Bill Otto), incubated overnight at 4°C. Appropriate secondary antibodies were used (Alexa-conjugated, Invitrogen, Yokohama, Japan) and the chromogen was developed with DAB.

The specimens were analyzed for the presence of ulcers/erosions, hyperplasic squamous epithelium (defined by the increase in the number of layers and presence of papillomatosis), metaplastic intestinal epithelium (defined by the presence of mucin-producing goblet cells and a mild architectural change extending upward from the anastomosis), and dysplastic intestinal epithelium (defined by pronounced architectural and cellular changes).[15] The sections were reviewed by 2 gastrointestinal pathologists (J.A. and K.T.). The immunofluorescent analysis was based on a semiquantitative count of positive cells at the anastomotic region, considering positive only the cells with a distinct nuclear expression of p53, CDX-2, PDX-1, and/or Ki-67.

Statistical analysis was performed with the SPSS package (SPSS Inc, Chicago, IL). Weight gain comparison was done with repeated-measures analysis of variance with Bonferroni correction (sphericity was violated but the differences were significant after Greenhouse-Geisser correction); rates of death, development of metaplasia and dysplasia, and PDX-1/CDX-2 staining were analyzed with the chi-square test.

Results

The mortality rate of EGJ was 13.0% (6/46), EJ was 11.6% (5/43), and EJ/TG was 13.0% (6/46). All 3 groups of operations had similar mortality rates, also comparable with reported studies using rats or mice.[6–8,16–23] All deaths in the EGJ group occurred in the first 30 days following the operation, in contrast with the other 2 groups, where they were more scattered along the follow-up period. When analyzing weight gain, the EGJ group showed gains equivalent to the control group, which was better than the EJ group and the EJ/TG group, in this order (Figure 2) ($P < .01$).

Because of technical complications, 7 paraffin specimens in the EGJ group and 1 paraffin specimen in the EJ/TG were lost, thus a total of 119 specimens were analyzed (EGJ, 33; EJ, 38; EJ/TG, 39; sham, 9). Macroscopically, the specimens showed thickening of the esophageal epithelium; ulcerations; and, rarely, nodulations (Figure 3).

Representative histologic findings are depicted in Figure 4, and the relative distribution of these findings according to the operation is shown in Table 1. Hyperplasic squamous epithelium was observed in 28/33 (84.8%) in the EGJ group, 28/38 (73.7%) in the EJ group, and 37/39 (94.9%) in the EJ/TG group. Metaplasia developed in 15/33 (45.5%) in the EGJ group and 6/39 (15.4%) in the EJ/TG

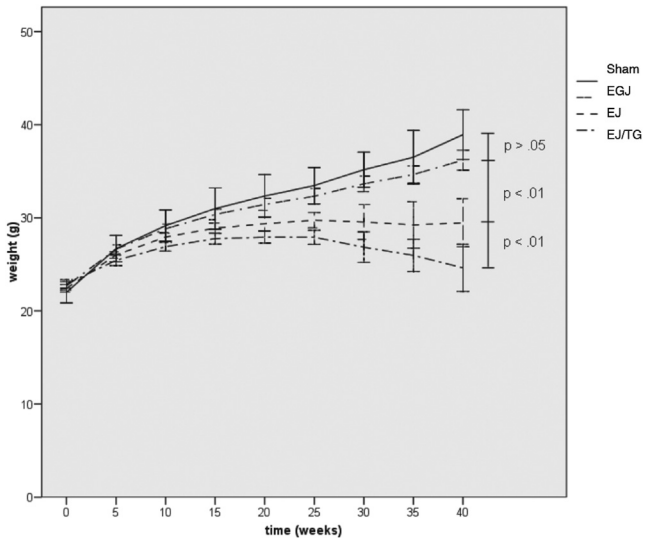

Figure 2. Weight gain curves grouped by the type of operation. There was no statistical difference between sham and EGJ ($P > .05$). However there were statistical differences between EGJ and EJ ($P < .01$), and between EJ and EJ/TG ($P < .01$).

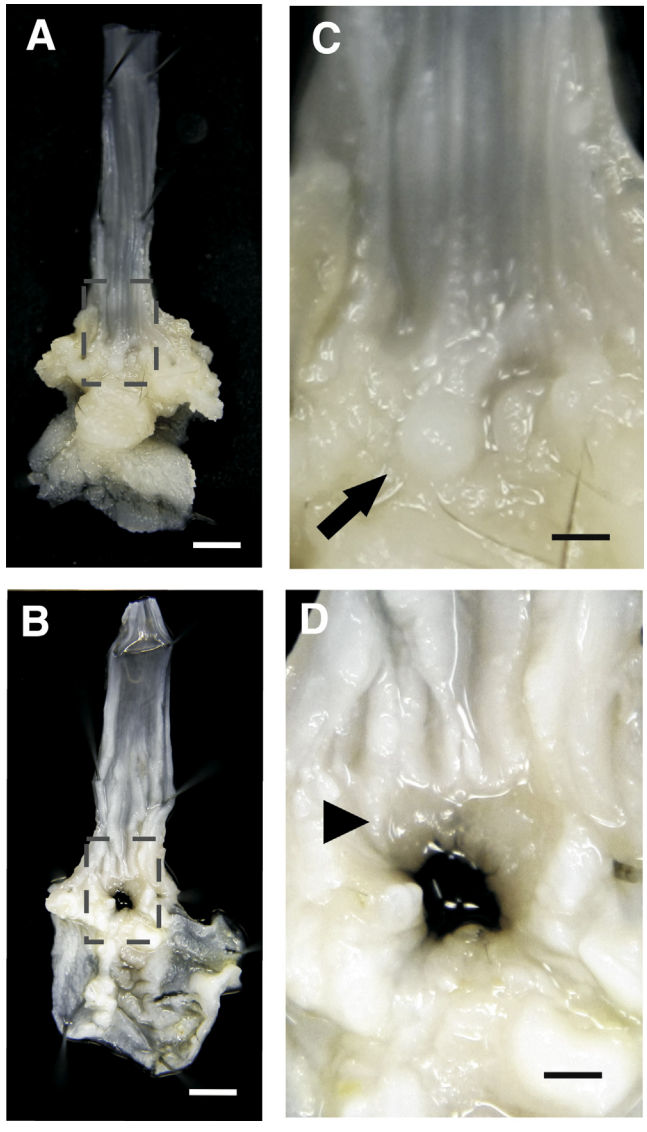

Figure 3. Macroscopic appearance of specimens. From EJ/TG operation (*A* and *C*) and EGJ operation (*B* and *D*). *Arrow* in *C* shows a nodular lesion and *arrowhead* in *D* shows an area of ulceration, surrounded by a thickened mucosa. Scale bars: 2 mm in (*A*, *B*); 0.5 mm in (*C*, *D*).

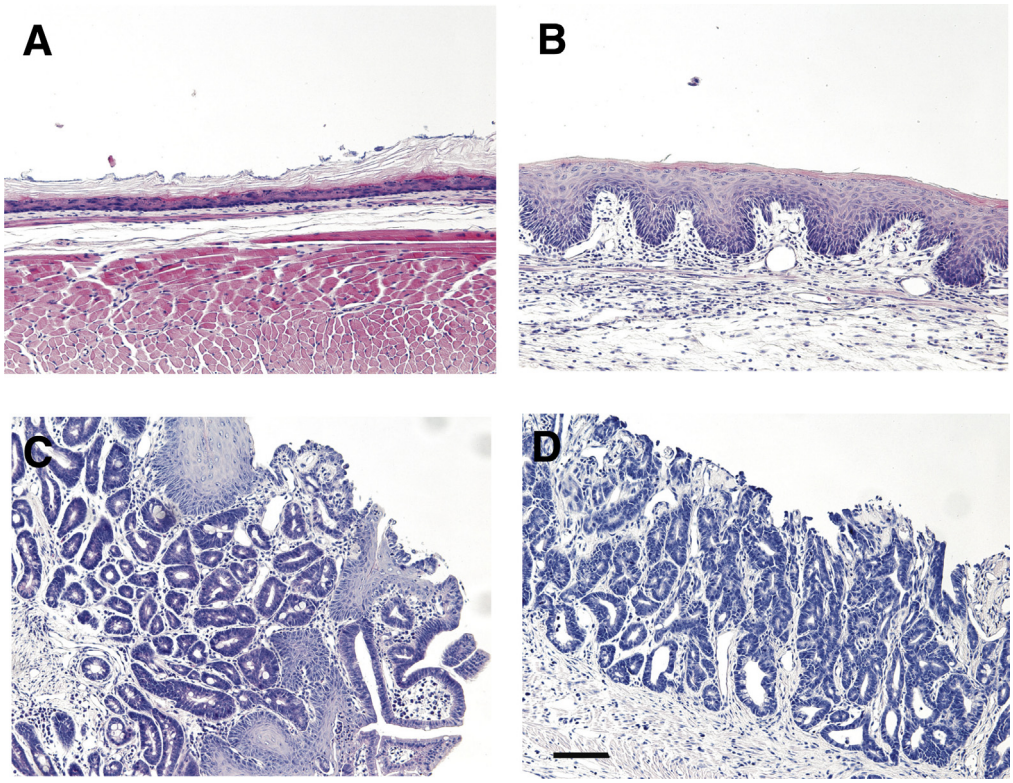

Figure 4. Representative histologic findings. (*A*) Normal squamous epithelium. (*B*) Hyperplasic squamous epithelium. (*C*) Metaplastic epithelium. (*D*) Dysplastic epithelium. Scale bar: 100 μm.

group, but no metaplasia developed in the EJ group (0/38) and in the control group (0/9). Dysplasia developed in 7/33 (21.2%) in the EGJ group and 1/39 (2.6%) in the EJ/TG group, but again no dysplasia developed in the EJ group (0/38) and in the control group (0/9). Comparing the 3 operations, EGJ showed significantly higher rates of metaplasia and dysplasia development.

Characteristics of Columnar Metaplasia

We next sought to define the characteristics of the columnar metaplasia observed in the reflux models. PAS/AB staining was positive in all the goblet cells of columnar metaplasia, consistent with an intestinal mucosal lineage profile. We therefore evaluated the expression of the intestinal master regulator transcription factor, CDX-2. Immunohistochemical studies showed a similar strong pattern of CDX-2 positivity in the metaplastic areas and adjacent normal intestinal epithelium, and there was no difference among surgical models. However, some dysplastic regions showed a decreased level of CDX-2 expression (50% of cases) and a different pattern of expression of the proliferative marker Ki-67 (75% of cases) (Figure 5). In dysplastic regions, Ki-67-positive cells were distributed in more surface area of the epithelium compared with nondysplastic metaplasia (Figure 5). TFF1 and TFF2 were negative in all the columnar metaplasia and dysplasia.

Previous investigations of reflux models have left concerns over whether the origin of the columnar metaplasia was simply invasion mucosa across the anastomosis. We therefore investigated the expression of the duodenal transcription factor PDX-1. PDX-1 was positive in 71% of metaplasia (15/21) and 88% of dysplasia (7/8), and its frequency was higher in dysplasia than in nondysplastic metaplasia (Figure 6). PDX-1 was expressed in 80% (12/15) of nondysplastic metaplasia in the EGJ group and 50% (3/6) of nondysplastic metaplasia in the EJ/TG group; in dysplasia, PDX1 was expressed in 85.7% (6/7) of cases in the EGJ group and 100% (1/1) of cases in the EJ/TG group. Because PDX-1 is not expressed in the normal jejunum, these findings suggest that columnar mucosa in the esophagus does represent a true metaplasia.

In dysplastic Barrett's epithelium in humans, upregulation of p53 staining is often considered one of the main characteristics of high-grade dysplasia.[24–28] p53 was positive in all of the dysplastic legions, 12/15 (80%) metaplasias in the EGJ model, and 1/6 (16.7%) metaplasia in the EJ/TG model (Figure 6). These results suggest that, especially in the case of the EGJ model, reflux elicits the formation of high-grade dysplasia within columnar metaplasia.

Table 1. Incidence of Histologic Findings According to the Types of Operation

	Hyperplasia	Metaplasia	Dysplasia
EGJ	28/33 (84.8)	15/33 (45.5)	7/33 (21.2)
EJ	28/38 (73.7)	0/38 (0)	0/38 (0)
EJ/TG	37/39 (94.9)	6/39 (15.4)	1/39 (2.6)
Sham	0/9 (0)	0/9 (0)	0/9 (0)

Number of specimens/total per group (%).

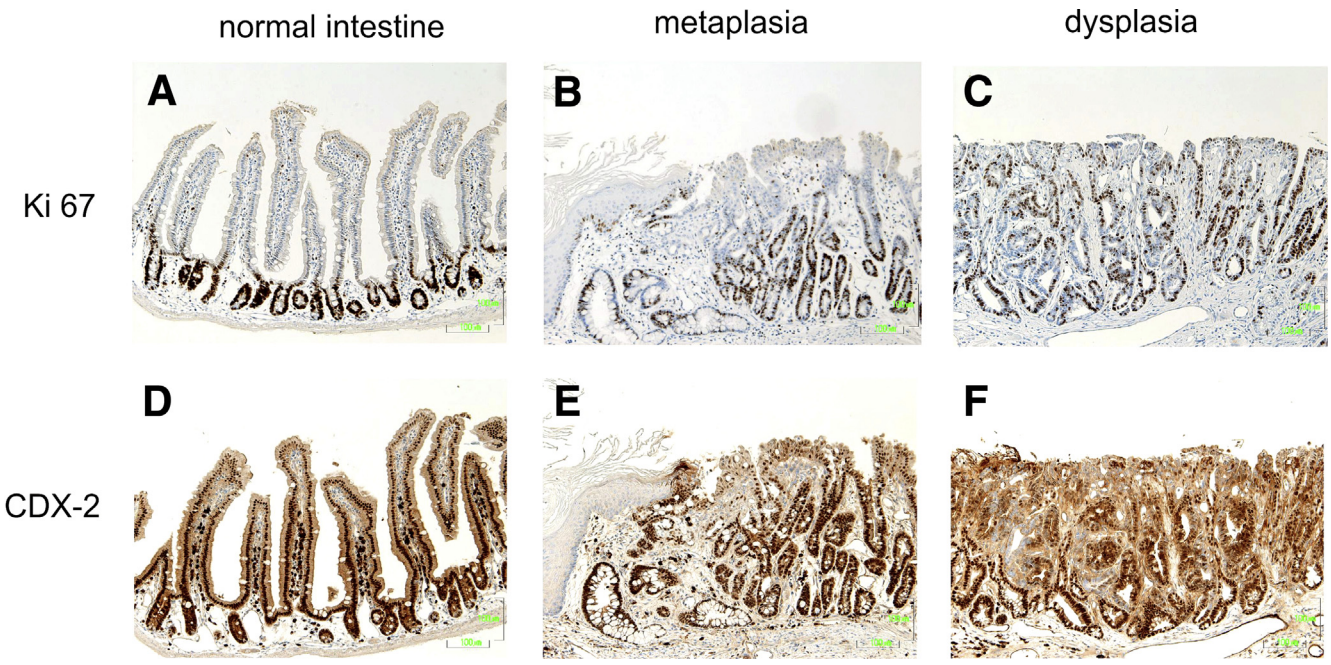

Figure 5. Comparison of Ki-67 and CDX-2. Ki-67 (*A–C*) and CDX-2 (*D–F*) expression in normal intestine (*A*, *D*), metaplastic (*B*, *E*), and dysplastic (*C*, *F*) esophageal columnar epithelium. Scale bar: 100 μm. CDX-2 is positive in all the cells in metaplasia but partly absent in dysplastic cells. Ki-67 was more scattered into mucosal surface cells in dysplasia.

Finally, in 1 case of note we observed the development of an island of intestinal metaplasia in the forestomach squamous mucosa opposite to the anastomotic orifice, confirmed by PAS/AB and CDX-2 staining (Figure 7). This case was in the EGJ group. This finding again demonstrates that columnar metaplasia can develop distant from the anastomosis with the small intestine.

Discussion

Differences Among Mouse Reflux Models

We have been able to develop mouse reflux models for esophagogastric junctional metaplasia and dysplasia with acceptable mortality. The occurrence rates of metaplasia in EGJ, EJ, and EJ/TG groups were 45.5%, 0%, and 15.4%, respectively, and the rate of dysplasia was 21.2%, 0%, and 2.6%, respectively. EGJ had the highest rate of histologic changes despite its partial reflux of biliopancreatic content, compared with the total reflux in the other models; this could be the result of an increased effect of alternating episodes of acid and alkali content in contact with the esophageal mucosa. In spite of having only alkaline reflux (without acid) the EJ/TG model had histologic changes, although with lower rates than the EGJ model. Finally, the reason for no effects in the EJ model, even considering its total reflux, may be that acid and alkali are neutralized before reaching the esophagus.

Comparison With Reported Mouse Models

In contrast to the multitude of rat studies, there are few published studies of mouse reflux models. Most of them have used an esophageal separation and EJ model and postoperative follow-up in the mice for around 20 weeks.[8,22,29] These studies reported the development of metaplasia in 14%–42% of cases and adenocarcinoma in 6% of cases. Our EGJ model developed metaplasia in 60.6% and dysplasia in 21.2% of cases and the rates were higher than previously reported models. One contrasting study by Raggi et al[30] showed an increased rate of development of metaplasia (60%) and adenocarcinoma (55%), using BALB/c mice. Other tested operations have included TG and EJ[23] and esophagoduodenostomy with or without TG,[31] all showing lower rates of metaplasia and adenocarcinoma. Pham et al[32] recently reported EJ model using C57Bl/6 mice. Their rates of metaplasia were 17% at 34 weeks and 7% by 52 weeks without development of carcinoma. They are lower than our EGJ model, but higher than our EJ model. We cannot explain these differences; however, reflux amount because of the sizes of the anastomosis might have affected the results. Finally, a recent study from our laboratory used an EGJ model and has demonstrated metaplasia in 45% of the mice after 40 weeks; no dysplasia or adenocarcinoma was found.[9] We do not know the reason for the lack of dysplasia in our former experiments. In this study, we compared mouse EGJ, EJ, and EJ/TG models and found that the EGJ model is the most efficient of the 3 models regarding the development of dysplasia. Most genetically modified mouse strains are made on a C57BL/C background, and thus the EGJ model should be the most suitable for these strains. The length of columnar metaplasia is short in mouse reflux models, and it is sometimes difficult to distinguish from anastomotic site of jejunum. The presence of PDX-1 is reported to indicate the existence of distinct pathways to metaplasia development, as suggested by Leys

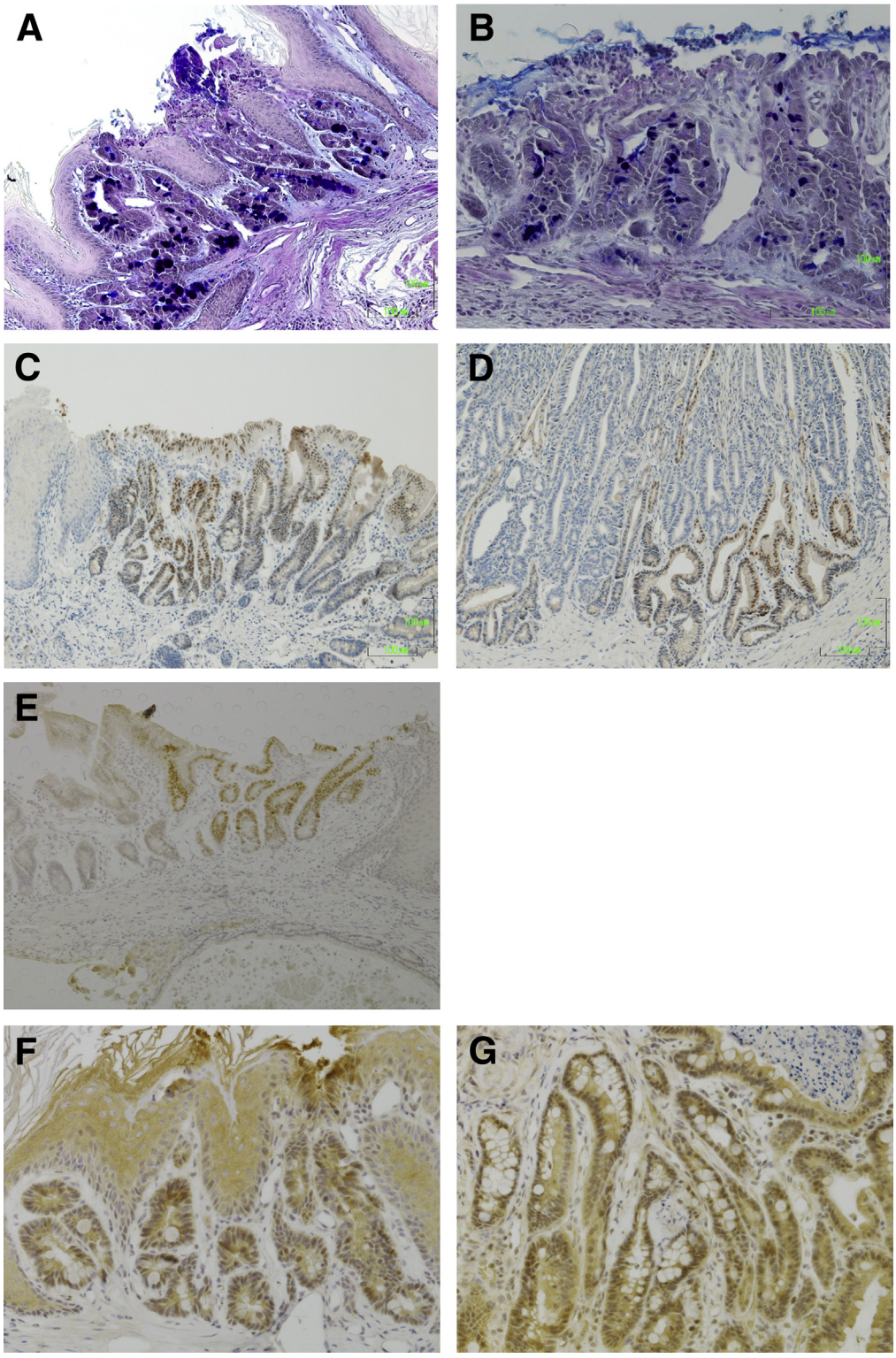

Figure 6. PAS/AB staining in metaplasia and dysplasia. PAS/AB staining in metaplasia (*A*), and in dysplasia (*B*). All the goblet cells are positive for PAS/AB staining. PDX-1 expression in metaplasia (*C*) and dysplasia (*D*), and negative expression in jejunum adjacent to columnar metaplasia in an EGJ model (*E*). PDX-1-positive cell rates were rather decreased in dysplastic change. P53 nuclear positivity in metaplasia (*F*), and dysplasia (*G*). Scale bar: 100 μm.

et al.[12] PDX-1 is expressed in normal gastric antrum, duodenum, and pancreas, without expression in the esophagus, gastric corpus, and jejunum. In our models, PDX-1 was positive in 71% of metaplasia and 88% of dysplasia. Not all the metaplasia and the dysplasia were positive for PDX-1; however, these metaplasia and dysplasia could be speculated to be true metaplasia for their ectopic homeobox gene expression.

Comparison With Rat Models

Most reflux models have been developed in rats.[6,7,16–21] In rats, high rates of hyperplasia developed as soon as the

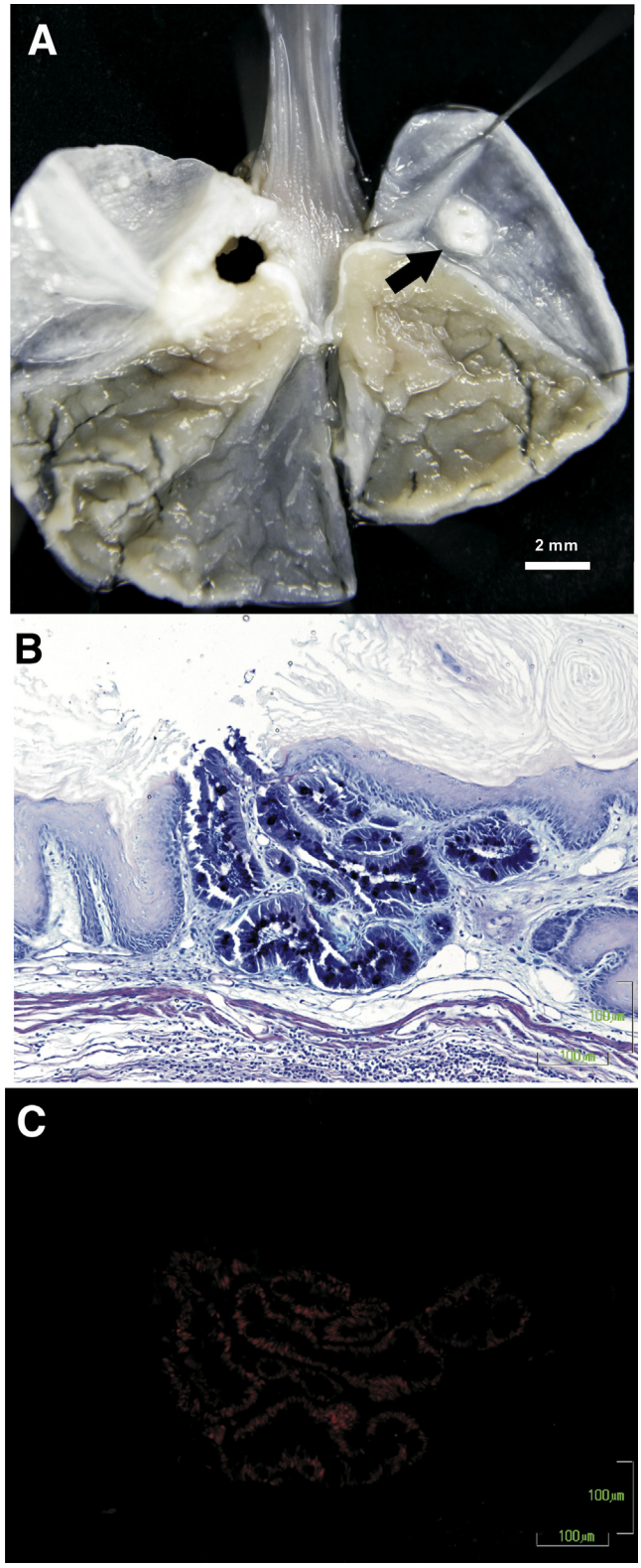

Figure 7. Induction of intestinal metaplasia in a segment of gastric forestomach squamous epithelium opposite to the anastomosis. (*A*) Macroscopic view, with *arrow* indicating the region of metaplasia. (*B*) PAS/AB staining in metaplasia. (*C*) CDX-2 immunofluorescence. Scale bars: 2 mm in *A*; 100 μm in *B* and *C*.

postoperative 10th week, followed by intestinal metaplasia by the 30th week (in 50%–100% of the cases), and finally adenocarcinoma developed around the 40th to 50th week (in 12%–75% of the cases). In this report, the highest frequency of metaplasia was obtained in the EGJ model: 60.6%, which is better than other mouse models, but worse than that of rat models. The reason for these differences may reflect a biologic difference between the species, because gastric intestinal metaplasia can be induced by sodium hydroxide treatment in rats,[33] but needs a genetically modified overexpression of CDX-2 under the promoter of H^+/K^+-ATPase to develop in mice.[34] Alternatively, metaplasia and dysplasia development may be related to the amount of the reflux, because a larger anastomotic orifice can be made in rats.

Etiologic Considerations

Junctional adenocarcinoma is reported to arise in Barrett's esophagus, columnar metaplasia, and in esophagogastric junction. Patients having Barrett's esophagus are followed with periodic endoscopy for early detection of adenocarcinoma, because biomarkers of dysplastic change of columnar metaplasia are lacking. In the mouse models reported here, this distinction is not resolved by the study of CDX-2 expression or by PAS/AB staining, because they merely reinforced the diagnosis of intestinal characteristics of these areas. In rats, Oh et al[35] demonstrated that a similar architectural characteristic was corroborated as intestinal metaplasia by an expression profile using trefoil peptides (TFF-1 and TFF-2). Using immunochemistry, we could not demonstrate an increased expression of these markers in our samples (not shown).

In our reflux models, EGJ model showed the best rates for metaplasia and dysplasia induction. The EGJ model causes reflux of both gastric juice and duodenal content without total mixture. In humans, with an intact stomach, gastric juice reflux can occur; however, duodenal content is difficult to reflux to esophagogastric junction without mixing with gastric juice.

One of the limitations of this study is that mouse reflux models have forced reflux of intestinal content and this is different from human physiological or pathologic reflux. Considerable controversy remains regarding the origin of columnar metaplasia in the esophagus. Some have suggested that in humans, these columnar metaplastic cells arise from esophageal submucosal glands. No such submucosal glands exist in rodents. Our results are most compatible with a migration of intestinal stem cells into the damaged squamous regions.[36] The columnar mucosal regions seem to represent true metaplastic lineages because they demonstrate characteristics distinct from jejunal mucosa including expression of the duodenal transcription factor PDX-1. It is also notable that more dysplastic lesions showed increased nuclear p53 staining, a hallmark of high-grade dysplasia in human Barrett's esophagus.[24–28]

In conclusion, in mice, the EGJ reflux model was the best to study the induction and progression of columnar metaplasia

in C57BL/6J mice. In this model, mice developed CDX-2 and PDX-1 expression metaplasia distant from the anastomotic site. Because high-grade dysplasia also expressed elevated nuclear p53, this model represents a relevant manipulation to study metaplastic progression in mice.

References

1. Pera M, Manterola C, Vidal O, Grande L. Epidemiology of esophageal adenocarcinoma. J Surg Oncol 2005; 92:151–159.
2. Botterweck AAM, Schouten LJ, Volovics A, Dorant E. Brandt PAvd. Trends in incidence of adenocarcinoma of the oesophagus and gastric cardia in ten European countries. Int J Epidemiol 2000;29:645–654.
3. DeMeester SR. Adenocarcinoma of the esophagus and cardia: a review of the disease and its treatment. Ann Surg Oncol 2005;13:12–30.
4. El-Serag HB. Time trends of gastroesophageal reflux disease: a systematic review. Clin Gastroenterol Hepatol 2007;5:17–26.
5. Shaheen N, Ransohoff D. Gastroesophageal reflux, Barrett esophagus, and esophageal cancer. JAMA 2002; 287:1972–1981.
6. Miwa K, Sahara H, Segawa M, Kinami S, Sato T, Miwazaki I, Hattori T. Reflux of duodenal or gastroduodenal contents induces esophageal carcinoma in rats. Int J Cancer 1996;67:269–274.
7. Fein M, Peters JH, Chandrasoma P, Ireland AP, Oberg S, Ritter MP, Bremner CG, Hagen JA, DeMeester TR. Duodenoesophageal reflux induces esophageal adenocarcinoma without exogenous carcinogen. J Gastrointest Surg 1998;2:260–268.
8. Xu X, III JL, Macri E, Loda M, Jr. FHE. Barrett's esophagus and associated adenocarcinoma in a mouse surgical model. J Surg Res 2000;88:120–124.
9. Silberg DG, Swain GP, Suh ER, Traber PG. Cdx1 and Cdx2 expression during intestinal development. Gastroenterology 2000;119:961–971.
10. Tatsuta T, Mukaisho K, Sugihara H, Miwa K, Tani T, Hattori T. Expression of Cdx2 in early GRCL of Barrett's esophagus induced in rats by duodenal reflux. Dig Dis Sci 2005;50:425–431.
11. Vallböhmer D, DeMeester SR, Peters J, Oh D, Kuramochi H, Shimizu D, Hagen JA, Danenberg KD, Danenberg PV, DeMeester TR, Chandrasoma PT. Cdx-2 expression in squamous and metaplasia columnar epithelia of the esophagus. Dis Esophagus 2006;19:260–266.
12. Leys CM, Nomura S, Rudzinski E, Kaminishi M, Montgomery E, Washington MK, Goldenring JR. Expression of Pdx-1 in human gastric metaplasia and gastric adenocarcinoma. Human Pathol 2006;37:1162–1168.
13. Wright N. The gastric epithelium: slow starter in the stem cell/lineage specification stakes? Cell Mol Gastroenterol Hepatol 2016;2:538–539.
14. ILAR. Guide for the care and use of laboratory animals. Washington: National Academy Press, 1996.
15. Petersen CP, Mills JC, Goldenring JR. Murine models of gastric corpus preneoplasia. Cell Mol Gastroenterol Hepatol 2017;3:11–26.
16. Bonde P, Sui G, Dhara S, Broor A, Kim IF, Wiley JE, Marti G, Duncan M, Jaffee E, Montgomery E, Maitra A, Harmon JW. Cytogenetic characterization and gene expression profiling in the rat reflux-induced esophageal tumor model. J Thorac Cardiovasc Surg 2007; 133:763–769.
17. Chen K-H, Mukaisho K-I, Sugihara H, Araki Y, Yamamoto G, Hattori T. 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;98:1683–1638.
18. Miyashita T, Ohta T, Fujimura T, Ninomiya I, Fushida S, Hattori T, Miwa K. Duodenal juice stimulates oesophageal stem cells to induce Barrett's oesophagus and oesophageal adenocarcinoma in rats. Oncol Rep 2006; 15:1469–1475.
19. Nishijima K, Miwa K, Miwashita 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 duodenoesophageal reflux in rats. Ann Surg 2004;240:57–67.
20. 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.
21. Buskens CJ, Hulscher JBF, Gulik TMV, Kate FJT, Lanschot JJV. Histopathologic evaluation of an animal model for Barrett's esophagus and adenocarcinoma of the distal esophagus. J Surg Res 2006; 135:337–344.
22. Ellis FH, Xu X, Kulke MH, LoCicero J, Loda M. Malignant transformation of the esophageal mucosa is enhanced in p27 knockout mice. J Thorac Cardiovasc Surg 2001;122: 809–814.
23. 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.
24. Murray L, Sedo A, Scott M, McManus D, Sloan JM, Hardie LJ, Forman D, Wild CP. TP53 and progression from Barrett's metaplasia to oesophageal adenocarcinoma in a UK population cohort. Gut 2006; 55:1390–1397.
25. Kaye PV, Haider SA, Ilyas M, James PD, Soomro I, Faisal W, Catton J, Parsons SL, Ragunath K. Barrett's dysplasia and the Vienna classification: reproducibility, prediction of progression and impact of consensus reporting and p53 immunohistochemistry. Histopathology 2009;54:699–712.
26. Kaye PV, Ilyas M, Soomro I, Haider SA, Atwal G, Menon S, Gill S, Richards C, Harrison R, West K, Ragunath K. Dysplasia in Barrett's oesophagus: p53 immunostaining is more reproducible than haematoxylin and eosin diagnosis and improves overall reliability, while grading is poorly reproducible. Histopathology 2016; 69:431–440.
27. Kastelein F, Biermann K, Steyerberg EW, Verheij J, Kalisvaart M, Looijenga LH, Stoop HA, Walter L,

Kuipers EJ, Spaander MC, Bruno MJ; ProBar-study group. Aberrant p53 protein expression is associated with an increased risk of neoplastic progression in patients with Barrett's oesophagus. Gut 2013; 62:1676–1683.
28. Weaver JM, Ross-Innes CS, Shannon N, Lynch AG, Forshew T, Barbera M, Murtaza M, Ong CA, Lao-Sirieix P, Dunning MJ, Smith L, Smith ML, Anderson CL, Carvalho B, O'Donovan M, Underwood TJ, May AP, Grehan N, Hardwick R, Davies J, Oloumi A, Aparicio S, Caldas C, Eldridge MD, Edwards PA, Rosenfeld N, Tavaré S, Fitzgerald RC; OCCAMS Consortium. Ordering of mutations in preinvasive disease stages of esophageal carcinogenesis. Nat Genet 2014;46:837–843.
29. Hutchinson L, Stenstrom B, Chen D, Piperdi B, Levey S, Lyle S, Wang TC, Houghton J. Human Barrett's adenocarcinoma of the esophagus, associated myofibroblasts and endothelium can arise from bone marrow derived cells after allogeneic stem cell transplant. Stem Cells Dev 2011;20:11–17.
30. Raggi M, Langer R, Feith M, Friess H, Schauer M, Theisen J. Successful evaluation of a new animal model using mice for esophageal adenocarcinoma. Langenbecks Arch Surg 2010;395:347–350.
31. 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.
32. 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;18:234–240; discussion 40–1.
33. Oohara T, Sadatsuki H, Kaminishi M, Mitarai Y. Simple alkaline treatment induces intestinal metaplasia in the stomach of rats. Pathol Res Pract 1982;175:365–372.
34. Mutoh H, Hakamata Y, Sato K, Eda A, Yanaka I, Honda S, Osawa H, Kaneko Y, Sugano K. Conversion of gastric mucosa to intestinal metaplasia in Cdx2-expressing transgenic mice. Biochem Biophys Res Commun 2002;294:470–449.
35. Oh DS, DeMeester SR, Dunst CM, Mori R, Lehman BJ, Kuramochi H, Danenberg K, Danenberg P, Hagen JA, Chandrasoma P, DeMeester TR. Validation of a rodent model of Barrett's esophagus using quantitative gene expression profiling. Surg Endosc 2009;23: 1346–1352.
36. Yamamoto Y, Wang X, Bertrand D, Kern F, Zhang T, Duleba M, Srivastava S, Khor CC, Hu Y, Wilson LH, Blaszyk H, Rolshud D, Teh M, Liu J, Howitt BE, Vincent M, Crum CP, Nagarajan N, Ho KY, McKeon F, Xian W. Mutational spectrum of Barrett's stem cells suggests paths to initiation of a precancerous lesion. Nat Commun 2016;7:10380.

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Fabio Terabe and Susumu Aikou, surgery for mice; Junko Aida and Kaiyo Takubo; pathologic analysis; Nobutake Yamamichi, immunohistochemistry; Michio Kaminishi and Yasuyuki Seto, study overview; and Sachiyo Nomura, research idea, analysis of the data, and manuscript preparation.

Conflicts of interest
The authors disclose no conflicts.

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