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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.

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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

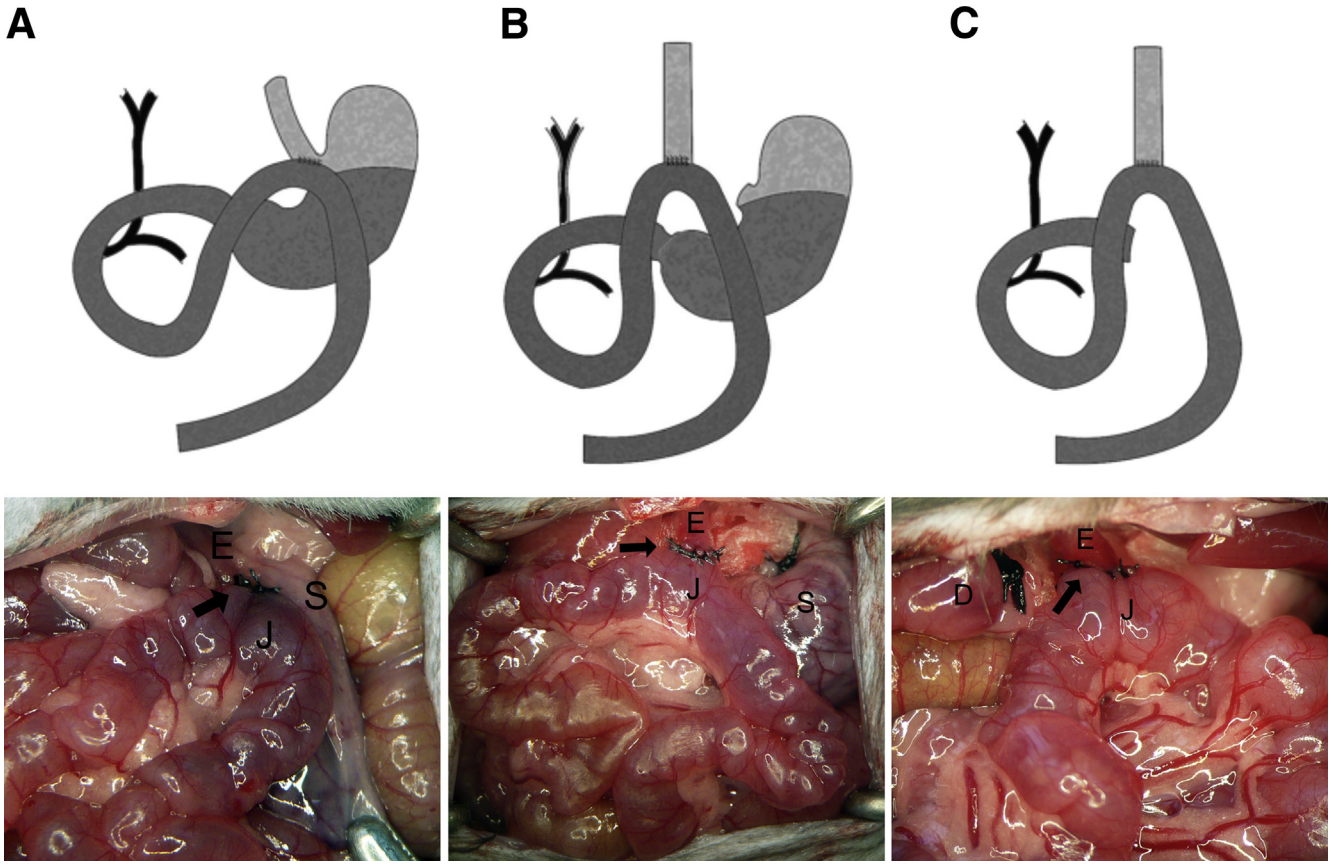

Figure 1. Types of operations. (*A*) EGJ. (*B*) Esophageal separation and EJ. (*C*) EJ/TG. EGJ has reflux of gastric content and intestinal content periodically. EJ has reflux of mixture of gastric content and intestinal content. EJ/TG has reflux of intestinal content without gastric acid. D, duodenum;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