Pathology International

Received: 10 November 2018 | Accepted: 22 March 2019

DOI: 10.1111/pin.12804

REVIEW ARTICLE

WILEY Pathology International

Barretts's carcinogenesis

Ken-ichi Mukaisho[1] | Shunpei Kanai[1] | Ryoji Kushima[2] | Takahisa Nakayama[1] | Takanori Hattori[1] | Hiroyuki Sugihara[1]

[1] Division of Molecular and Diagnostic Pathology, Department of Pathology, Shiga University of Medical Science, Otsu, Japan

[2] Division of Diagnostic Pathology, Shiga University of Medical Science Hospital, Otsu, Japan

Barrett's esophagus is considered a precancerous lesion of esophageal adenocarcinoma (EAC). Long-segment Barrett's esophagus, which is generally associated with intestinal metaplasia, has a higher rate of carcinogenesis than short-segment Barrett's esophagus, which is mainly composed of cardiac-type mucosa. However, a large number of cases reportedly develop EAC from the cardiac-type mucosa which has the potential to involve intestinal phenotypes. There is no consensus regarding whether the definition of Barrett's epithelium should include intestinal metaplasia. Basic researches using rodent models have provided information regarding the origins of Barrett's epithelium. Nevertheless, it remains unclear whether differentiated gastric columnar epithelium or stratified esophageal squamous epithelium undergo transdifferentiation into the intestinal-type columnar epithelium, transcommittment into the columnar epithelium, or whether the other pathways exist. Reflux of duodenal fluid including bile acids into the stomach may occur when an individual lies down after eating, which could cause the digestive juices to collect in the fornix of the stomach. *N*-nitroso-bile acids are produced with nitrites that are secreted from the salivary glands, and bile acids can drive expression of pro-inflammatory cytokines via EGFR or the NF-κB pathway. These steps may contribute significantly to carcinogenesis.

KEYWORDS

Barret's esophagus, bile acid, gastroesophageal reflux, metaplasia

Correspondence:
Ken-ichi Mukaisho, MD, PhD, FIAC, Division of Molecular and Diagnostic Pathology, Department of Pathology, Shiga University of Medical Science, Seta-tsukinowa-cho, Otsu, 520-2192 Shiga, Japan.
Email: mukaisho@belle.shiga-med.ac.jp

EPIDEMIOLOGY OF ESOPHAGEAL CANCER

Histological studies have revealed that the main epithelial malignant tumors of the esophagus are esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC), which have different risk factors.[1] The primary risk factor for EAC is gastroesophageal reflux disease (GERD).[2–4] GERD is a major cause of reflux esophagitis, which occurs when gastric acid, bile acids and other harmful substances in gastric juice flow backward into the esophagus,[5] which is generally covered with stratified squamous epithelium. Most esophageal cancers in Asian countries,

including Japan, involve ESCC.[6,7] In contrast, Western countries have recently seen a sharp increase in the prevalences of EAC and esophagogastric junction cancer.[8] In the US, EAC accounts for approximately 60% of all esophageal cancers, and ESCC had historically been predominant, although EAC appears to have overtaken ESCC in approximately 1995.[7,9,10] This change may be related to the sharp increase in the number of obese individuals with a high-fat diet and the resulting increase in the prevalence of GERD.[7,8,11,12] Japan has also seen an increase in the prevalence of GERD, in conjunction with increasingly Westernized dietary habits, a reduced rate of *Helicobacter pylori* (*H. pylori*) infection and an expanding elderly population.[13–21] Thus, there are fears that the prevalence of EAC will continue to gradually increase.[22]

DEFINITION OF BARRETT'S ESOPHAGUS

Barrett's epithelium is metaplasia from squamous epithelium into columnar epithelium,[23] with esophageal Barrett's epithelium being known as Barrett's esophagus.[24] Barrett's esophagus is considered a precancerous lesion of EAC, although there is no universally accepted definition of Barrett's esophagus.[25] The key factor in defining Barrett's esophagus involves the presence of goblet cells, and there is no disagreement that Barrett's esophagus involves a columnar epithelium. The American definition of Barrett's epithelium only considers intestinal metaplastic mucosa with goblet cells (Fig. 1a), which has led to the use of the phrase 'no goblets, no Barrett's'.[26–28] In Germany, the histological diagnosis of Barrett's esophagus still requires the proof of a specialized intestinal metaplastic epithelium (columnar epithelium with goblet cells).[29] In contrast, the Japanese definition of Barrett's mucosa is 'columnar epithelium continuous from the stomach with or without intestinal metaplasia'[24] (Fig. 1b). The British Society of Gastroenterology also defines this condition as 'an esophagus in which any portion of the normal distal squamous epithelial lining has been replaced by metaplastic columnar epithelium and includes a ≥ 1 cm segment length criterion', regardless of whether goblet cells are present.[30]

WHERE IS THE ESOPHAGOGASTRIC JUNCTION?

In the UK, a ≥ 1-cm segment of columnar epithelium fulfills the definition of Barrett's esophagus,[30] with Barrett's epithelium being classified based on its length into short-segment Barrett's esophagus (SSBE; a < 3-cm segment) and long-segment Barrett's esophagus (LSBE; a ≥ 3-cm segment).[24] Thus, the distance from the esophagogastric junction is key to defining and classifying Barrett's esophagus, although this must be based on the precise location of the esophagogastric junction. In Western countries, the esophagogastric junction is endoscopically defined as the upper margin of the gastric mucosal folds (the oral margin of the longitudinal folds of the greater curvature of the stomach), whereas in Japan the esophagogastric junction is defined as the lower end of the palisade vessels.[31–35] The histological requirements of this definition are the presence of (i) esophageal gland ducts in the mucosal layer or proper esophageal glands in the submucosal layer within the area of columnar epithelium;[36,37] (ii) squamous islands in the columnar epithelium;[38] and (iii) a double-layer muscularis mucosae.[37] Recently, palisade vessels have also been reported as a new histologic marker of esophageal origin.[39]

LENGTH OF BARRETT'S ESOPHAGUS AND CARCINOGENESIS

In the US, 5.6% of adults have LSBE and 10 to 15% of adults have SSBE.[12,40] In Japan, the rates are approximately 17.9%

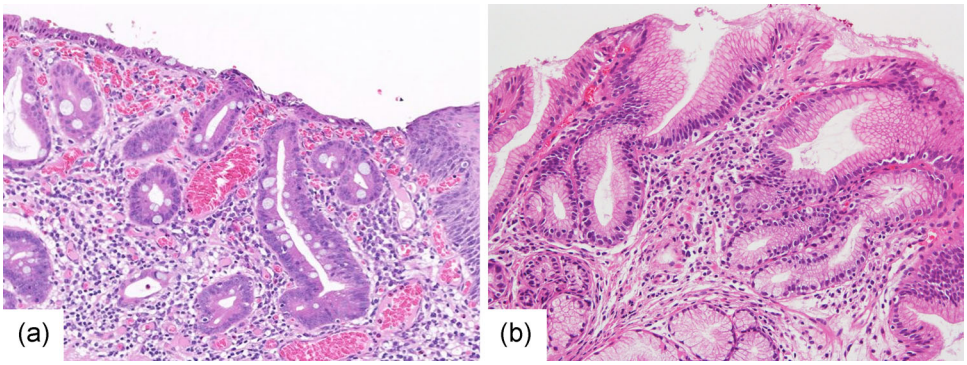

Figure 1 Definition of Barrett's esophagus. **(a)** Intestinal metaplasia with goblet cells. **(b)** Columnar epithelium without goblet cells. The American and German definition of Barrett's epithelium only considers intestinal metaplastic mucosa with goblet cells **(a)**. In contrast, the Japanese and British definition of Barrett's mucosa does not require goblet cells **(b)**. However, in the British Society of Gastroenterology includes a ≥ 1 cm segment length criterion.

(1.2–59.0%) for SSBE and approximately 0.4% (0.2–1.4%) for LSBE,[41] with SSBE also being more common in other Asian countries.[42–44] Barrett's esophagus is thought to have an annual carcinogenesis rate of 0.15 to 0.65%.[45,46] Other reports have also examined the carcinogenesis risk of Barrett's esophagus, with some reports attributing similar risks to SSBE and LSBE[47] and other recent reports indicating the LSBE has a greater carcinogenesis risk than SSBE.[48,49] One recent report from Japan focusing on follow-up of patients with LSBE revealed a carcinogenesis rate of approximately 1.2% per year (3 cancers/251 cases/year; 12 cases per 1000 people),[50] which is higher than the reported rates from other countries. This discrepancy may be related to the small sample size of the Japanese study, although it could also be attributed to the different diagnostic criteria for adenocarcinoma in Japan and Western countries. The Vienna classification was published by a global group of gastrointestinal pathologists to unify the diagnostic criteria for tumors of the gastrointestinal tract,[51] although it is practically difficult to achieve this unification. For example, some intramucosal atypical gland-forming lesions are diagnosed as EAC in Japan, even in cases that would be diagnosed as low-grade or high-grade dysplasia in Western countries. This difference is related to Western countries defining carcinoma as involving invasion, while the Japanese definition is based on nuclear and structural atypia. Therefore, in Western countries, most mucosal lesions with nuclear or structural atypia (except poorly-differentiated adenocarcinoma or undifferentiated tumors, such as signet ring cell carcinoma) would be classified as dysplasia and not cancer.

BARRETT'S EPITHELIUM: HISTOLOGICAL TYPE AND CARCINOGENESIS

The designation Barrett's esophagus arises from Barrett's report describing 'part of the foregut, distal to the cricopharyngeal sphincter, which is lined by squamous epithelium'.[52] The report also notes that 'He commented on earlier reports describing patients with ulcerations in a tubular organ that grossly appeared to be the esophagus but had a distal, ulcerated portion lined by columnar epithelium'.[53] At the time, it was considered a form of the stomach that was congenitally present in the esophagus, which was referred to as 'short esophagus'.[52,53] The term 'Barrett's esophagus' was not initially used. Various reports subsequently provided histological images of Barrett's esophagus, with Paul *et al*.[54] presenting images from 11 cases of Barrett's esophagus in 1976, which they concluded showed three types of columnar epithelium in the lower esophagus: atrophic gastric mucosa (now 'oxyntic mucosa'), junctional mucosa (now 'cardiac-type mucosa'), and specialized columnar epithelium ('intestinal metaplasia'). This report conflicts with the current American diagnostic criteria for Barrett's epithelium, which emphasize intestinal metaplasia, while reports from the 1980s implicated intestinal metaplasia in the development of Barrett's esophagus and various epithelial cancers, although intestinal metaplasia was considered essential to the definition of Barrett's esophagus (a precancerous lesion).[55–60] As intestinal metaplasia is often seen as a histological type of LSBE, intestinal metaplasia could also be considered essential for carcinogenesis in the US. However, the concept that intestinal metaplasia is required for cancer to develop has become a sort of dogma in the US. The choice of the word "dogma" can be better understood by considering the example of gastric carcinogenesis. It is common knowledge that gastric cancer is often caused by *Helicobacter pylori* infection, which causes superficial gastritis to become chronic active gastritis, and chronic atrophic gastritis subsequently results in intestinal metaplasia leading to gastric cancer (especially differentiated gastric cancer).[61,62] In the US, research revealed that intestinal metaplasia was commonly found in the background mucosa of gastric cancer, before the discovery of *H. pylori*, which led to the belief that intestinal metaplasia was a precancerous lesion leading to gastric cancer.[63,64]

IS IT TRUE THAT 'ONLY INTESTINAL METAPLASIA RESULTS IN CANCER'?

The process of esophageal and gastric carcinogenesis raises questions regarding the hypothesis that 'only intestinal metaplasia results in cancer'. For example, Hattori's 1985 study investigated mucous granules from gastric cancer cells occurring in hyperplastic polyps without any intestinal metaplasia and revealed that gastric cancer developed from intrinsic gastric glands.[65] We also recently reported that gastric cancer is also observed developing with hyperplastic polyps in the background and proved the genomic change in this intramucosal lesion.[66] Subsequent research has aimed to study the mucous phenotypes of gastric cancer and its background mucosa.[67–71] In this context, gastric cancer reportedly occurs from intrinsic gastric glands that have atrophied with sufficiently intense inflammation to cause intestinal metaplasia.[72] It means that intestinal metaplasia is not always a 'precancerous' lesion but a 'paracancerous' lesion. In our recent report, we found that early intramucosal gastric cancer lesions possessed gastric mucous phenotypes and that there is a proliferative zone with a polarity of differentiation in mucous phenotypes that is similar to the intrinsic gastric glands.[73] Thus, it does not appear that intestinal metaplasia is a major oncogenic pathway for gastric cancer. This gland-forming differentiated adenocarcinoma with gastric mucous phenotype is finally gaining global recognition, and even the widely accepted 2011 WHO system classifies gastric phenotype neoplasia.[74]

SOME EACS DEVELOP FROM GASTRIC-TYPE MUCOSA

An analysis of EACs reported in 2009, using German cases, revealed that small EACs do not exclusively develop from intestinal metaplasia, and can develop from gastric cardiac-type mucosa.[75] A report from the UK has also stated that intestinal metaplasia is not a universally-present component of oncogenesis.[76] Columnar metaplasia without goblet cells can involve abnormal DNA. Results from the other studies of EAC also revealed that these tumors were often gastric phenotype.[77,78] These results confirm that EAC development is not restricted to the intestinal metaplasia. Columnar metaplasia without goblet cells also reportedly has the potential to involve intestinal phenotypes.[79–82] In German cases, approximately 60% of Barrett's mucosa on the background of EAC in the lower esophagus was cardiac-type mucosa without goblet cells, although the glands often had intestinal phenotypes (versus pure gastric phenotypes) with the expression of intestinal phenotypes claudin 3 and claudin 4.[77] Epidemiological data indicate that LSBE, which is often associated with intestinal metaplasia, has a higher carcinogenesis rate than SSBE, and that cases with a background of intestinal metaplasia often have intense inflammation and severe reflux esophagitis. Thus, Barrett's epithelium involving intestinal metaplasia is likely to involve more intense reflux-based irritation, and it might be understood that Barrett's epithelium with intestinal metaplasia involves a higher risk of carcinogenesis than if intestinal metaplasia was absent.

RELATIONSHIP BETWEEN BARRETT'S EPITHELIUM AND GASTRODUODENAL REFLUX

A previous report in 1979 indicated that Barrett's esophagus developed after total gastrectomy in humans, which suggested that gastric acid reflux was not necessary for Barrett's esophagus to occur.[83] It is now widely accepted that reflux of both gastric acid and bile acids must be involved for Barrett's epithelium to occur.[84] To evaluate the histogenesis of Barrett's epithelium, many researchers, including our group, have used rat models of surgically induced gastroduodenal reflux that causes reflux of duodenal fluid, including bile acids, with or without gastric juice, into the esophagus[85–88] (Fig. 2). These models have revealed that, even in the absence of carcinogenic agents, Barrett's epithelium with intestinal metaplasia developed 10 to 20 weeks after surgery, and dysplasia and EAC could be observed after approximately 40 weeks[85–88] (Fig. 3). Thus, reflux of duodenal fluid, including bile acids, is essential for Barrett's epithelium with goblet cells to occur. In contrast, reflux of gastric juice without duodenal fluid only induces light esophagitis and does not appear to produce Barrett's esophagus with intestinal metaplasia.[77] *In vitro* experiments have indicated that exposure to bile acids can induce the expression of CDX1 and CDX2, which are transcription factors that are involved in the production/maintenance of the intestinal epithelium.[89–91] Therefore, bile acid reflux has been implicated as being involved in the occurrence of Barrett's esophagus with intestinal metaplasia.

DEVELOPMENT OF BARRETT'S EPITHELIUM

Many hypotheses have been reported regarding the cells from which Barrett's mucosa originates. These include (i) the columnar epithelium being directly produced from the esophageal squamous epithelium[87,89,90] (Fig. 4a); (ii) the gastric mucosa[77,91] (Fig. 4b); (iii) the esophageal gland duct[92] (Fig. 4c); (iv) the esophagogastric junction mucosa[93] (Fig. 4d); (v) the fetal remnant[94] (Fig. 4e,f); (vi) bone marrow cells[95] (Fig. 4g); and (vii) wound repair.[96] Most of these hypotheses have been developed based on results from rat- or mouse-based models. The genetic

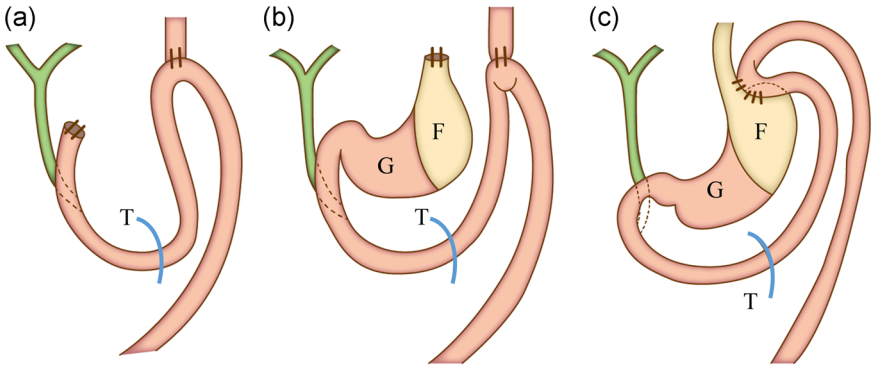

Figure 2 Various rat reflux models of Barrett's esophagus leading to adenocarcinoma. F, forestomach; G, glandular stomach; T, treitz ligament. **(a)** Esophago-jejunostomy after total gastrectomy.[86,87] **(b)** Esophago-jejunostomy without gastrectomy.[87,88] **(c)** The esophagogastric junction was side-to-side anastomosed to a loop of jejunum.[93]

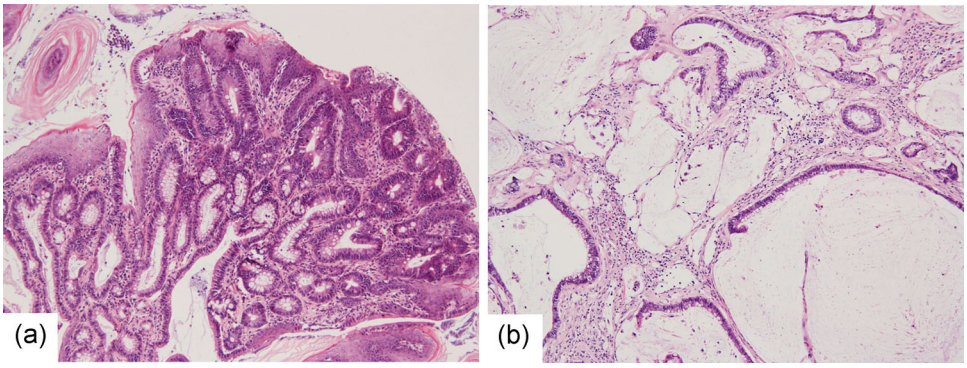

Figure 3 Neoplastic lesions developed in rat reflux models. **(a)** Dysplasia. **(b)** Mucinous adenocarcinoma.

changes have typically been evaluated in mice, because of their well-understood genome. However, mice and rats differ anatomically from humans, as they lack esophageal glands, which makes it impossible to determine whether Barrett's mucosa develops from these glands. Furthermore, rats lack a gall bladder, and the surgically induced model causes continuous bile reflux based on the absence of a gallbladder. Pigs have also been used to study whether the esophageal glands are involved in the occurrence of EAC from Barrett's epithelium. It is impossible to definitively identify the correct theory and research in this field is expected to continue. In the following sections, we will introduce the process based on the findings from our rat gastroduodenal reflux models.[77,87,88] There are at least two ways for Barrett's epithelium to develop.[77]

Development from the basal layer of the esophageal squamous epithelium

In the rat reflux models, columnar epithelium was observed in the basal layer of the squamous epithelium, which involved regenerative changes caused by inflammation at locations

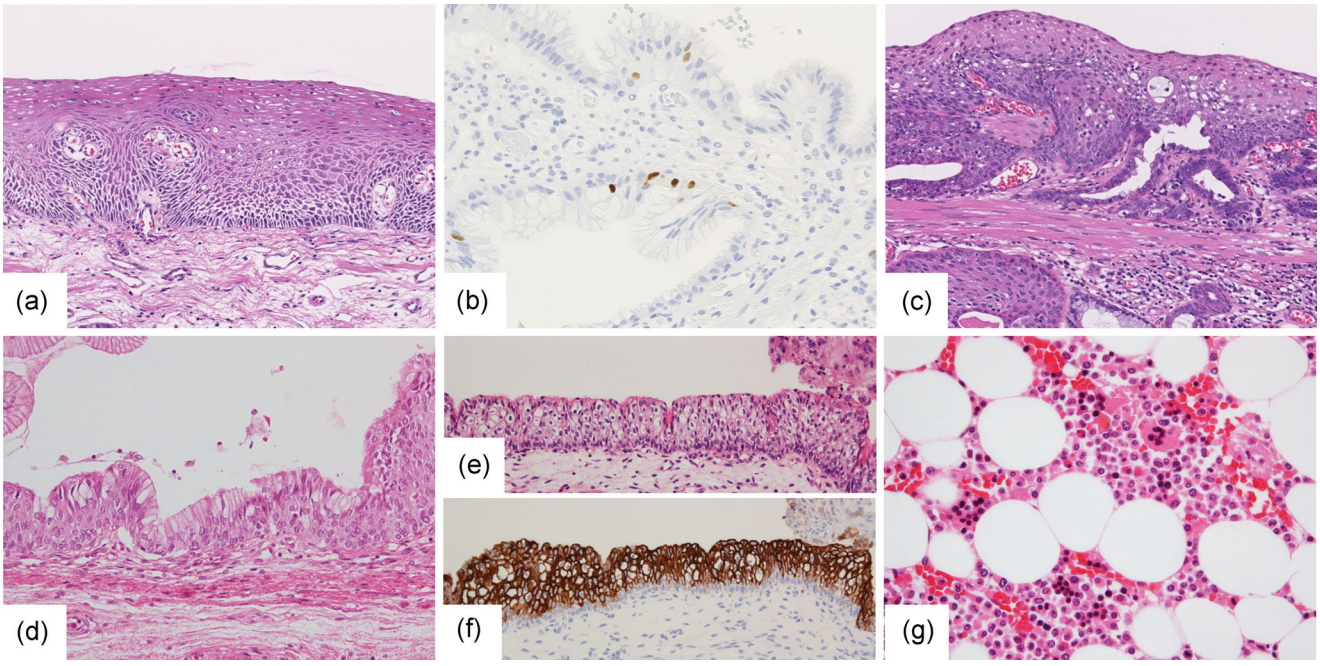

Figure 4 Various candidate lesions about development of Barrett's epithelium. **(a,c–e,g)** HE stainings. (**b**) CDX2. (**f**) CK7 Visualization of immunochemical staining was performed using 3,3′-diaminobenzadine (DAB). **(a)** Inflamed esophageal squamous epithelium. **(b)** Gastric mucosa (cardiac-type mucosa) focally positive for CDX2 expression has the potential for intestinal differentiation. (Brown is the color of positive cells). **(c)** Esophageal gland duct. **(d)** Esophagogastric junction mucosa. **(e,f)** Twenty-two-week-old human fetal esophagi. The epithelium has cilia, and it is positive for CK7. **(f)** Bone marrow.

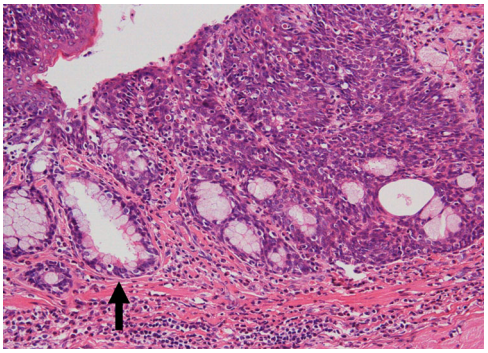

Figure 5 Histological image of a site distant from the anastomotic region in a rat model that underwent esophago-jejunostomy. Several columnar epithelia have developed within the squamous epithelium, as shown on the right side of the figure, while complete Barrett's epithelium (arrow) can be seen on the left side of the figure.

distant from the anastomosis (Fig. 5). This process cannot be attributed to transdifferentiation (i.e., completely differentiated squamous epithelium differentiating into columnar epithelium) and must be attributed to transcommitment, in which stem cells located in the basal layer of the stratified squamous epithelium change their direction of differentiation from squamous to columnar epithelium. In a review on reflux esophagitis by Souza, an alternative concept to the conventional theory of 'reflux esophagitis is an acid burn' is proposed. According to this concept, 'the reflux of acid and bile salts does not destroy epithelial cells directly, but rather induces them to secrete pro-inflammatory cytokines'. Columnar epithelium that exists in the squamous epithelial basal layer is thought to develop as a result of cytokine sizzle.[97] One recent report has described a non-neoplastic cell line established from the esophageal stratified squamous epithelium of a patient with GERD who experienced bile acid reflux for approximately 5 min daily over a 30-week period. That cell line exhibited expression of TAp63, CDX2 and SOX9, similar to cell lines established from Barrett's epithelium, as well as similar morphological changes.[98] They suggested that the non-neoplastic cell line, which was derived from a biopsy specimen of a patient with GERD, may be derived from preterminal parent cells that retain the proliferative capacity and may not represent true 'fully terminally differentiated' epithelial cells. Considering their precursor-like properties, this behavior is more synonymous with reprogramming or transcommitment rather than transdifferentiation.[98] They claimed that these biphenotypic progenitors may be the precursors for the Barrett's columnar epithelium.[98]

Development from the cardiac-type mucosa

This section addresses the development of cardiac-type mucosa, which is commonly associated with SSBE in Japan. Results from our animal models, which mimic human gastroduodenal reflux through the esophagogastric junction (Fig. 6), have suggested that cardiac-type mucosa forms Barrett's mucosa by spreading to the oral side, which replaces the place where esophageal stratified squamous epithelium is missing because of erosion or ulceration[77] (Fig. 7). This is so-called 'creeping theory', which involves stem cells around the missing epithelium gradually spreading to cover the defect without overt proliferative activity. Thus, if reflux esophagitis is not treated (e.g., by using a proton pump inhibitor (PPI)), there is a persistent cause of the defect in the esophageal mucosa. Furthermore, with sustained reflux, the cardiac glands spread to the oral side faster than the oral-side squamous epithelium can repair the defect. The squamous epithelium is also more vulnerable to gastric acid or bile than the gastric columnar epithelium, which makes it impossible to restore the missing epithelium if there is sustained reflux. In contrast, endoscopic images from cases treated using a PPI had frequently revealed signs that the defect sites from before PPI therapy were repaired by stratified squamous epithelium. Therefore, it appears that

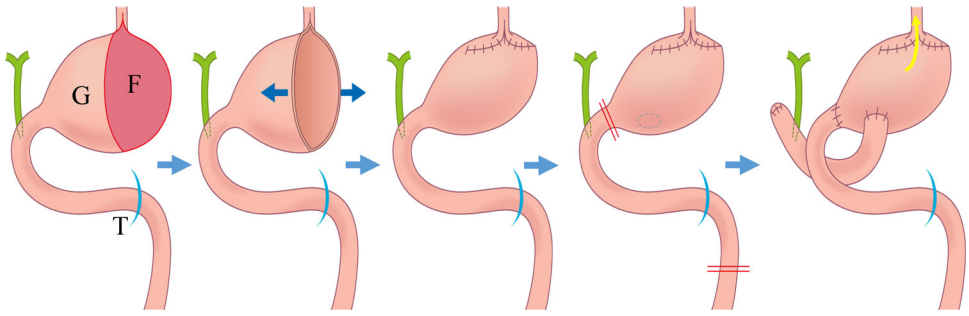

Figure 6 Surgical procedure of the rat model which mimics human gastroduodenal reflux: F, forestomach; G, glandular stomach; T, treitz ligament. Surgery proceeds from left to right. The final phase is shown on the extreme right. Firstly, we removed the forestomach which does not exist in the human stomach. Then, the esophagus was connected with the glandular stomach. The gastrointestinal tract is sectioned where the red double line is drawn in the second figure from the right and then anastomosed. In the final phase, the reflux of gastric fluid that contains duodenal fluid occurs from the glandular stomach to the esophagus (yellow arrow).

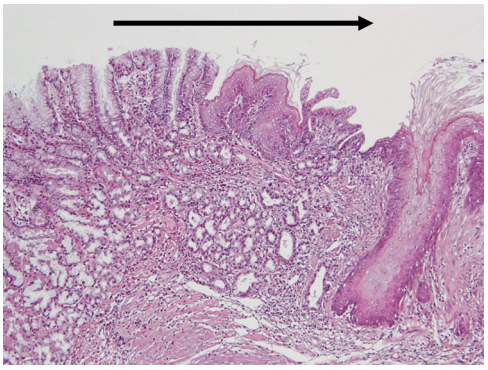

Figure 7 Cardiac-type mucosa around the esophagoglandular-stomach anastomosis developed in the rat reflux model. The right side of the figure is an oral side. Expansion of intrinsic gastric cardiac glands occurs to cover the defect of squamous epithelium during wound repair (arrow).

replacement of the missing mucosal epithelium by stratified squamous epithelium or columnar epithelium depends on the local environment, which is related to the presence or absence of sustained reflux.

Summary of the development of Barrett's mucosa

Based on the results of studies that used rat reflux models, at least two pathways are involved in the development of Barrett's esophagus. We believe SSBE development can be explained by spreading of a cardiac-type mucosa to the oral side due to wound repair, while LSBE development can be explained by the presence of progenitor cells or stem cells in the basal layer of the esophageal stratified squamous epithelium transcommitment, as a result of cytokine sizzle. These two pathways may be simultaneously involved in human cases. A developmental process that involves the esophageal glands may also exist that cannot be verified using rats, which have no esophageal glands.

BARRETT'S CARCINOGENSIS

Effects of a high-animal-fat diet

Although Humans who have developed Barrett's esophagus reportedly had greater bile acid reflux in the esophagus,[99] it remains unclear which bile acids are involved in carcinogenesis from Barrett's esophagus. Nehra *et al*.[99] have reported that significant amounts of taurine-conjugated bile acids were detected in the esophagi of patients who had developed Barrett's esophagus. In the US, the increase in the incidence of Barrett's esophagus is reportedly linked to the consumption of a high-fat diet,[8] and our rat model has shown that a high-fat diet consisting mainly of tallow increases the proportion of taurine-conjugated bile acids.[100] The pH in the stomach and the acid dissociation constant of each bile acid need to be considered. The pKa value of taurine conjugates is strikingly lower at approximately 1.8 to 1.9 and taurine conjugates do not form deposits,[101] even in cases with bile acid reflux into the stomach.

Activation of NF-κB

In vitro experiments also indicated that taurine-conjugated bile acids activate Src, EGFR and ERK, thereby causing colorectal cancer cells to proliferate.[102] Activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which is reportedly overexpressed after exposure to bile acids, has also been implicated in carcinogenesis from Barrett's esophagus.[103] It has also been reported that acid and bile acids caused activation of NF-κB.[104,105] Bile acids are known to increase CDX1 and CDX2 expression and promote differentiation into intestinal epithelium.[89,106–110] CDX2 is targeted by the NF-κB pathway,[111,112] and two putative NF-κB binding sites have been identified in the CDX2 promoter.[113] Therefore, digestive juice reflux induces the production of cytokines that are involved in Barrett's carcinogenesis. This carcinogenic process is likely initiated by a factor that is produced in the patient's body, and we will cover some potential factors below.

Production and stabilization of *N*-nitroso-bile acids

N-nitroso-compounds have been implicated in the occurrence of gastric cancer,[114] and endogenous *N*-nitroso-bile acids are one kind of nitroso compounds. Although bile acid itself is not mutagenic, *N*-nitroso-bile acids are mutagenic and are produced if nitrite or nitrate and bile acid are present in acidic conditions. Based on our reflux models, we found that administration of L-thioproline (which plays a role in capturing nitrite) suppressed the occurrence of esophageal or gastric cancer, which indirectly shows that *N*-nitroso-bile acids are involved in the occurrence of esophageal and gastric cancers.[115,116] DNA adducts originating from *N*-nitroso-bile acids have also been detected in the glandular stomach from our gastric cancer model.[117,118] Furthermore, *N*-nitroso-bile acids reportedly stabilize under acidic conditions.[119] These findings suggest that *N*-nitroso-bile acids appear to be involved in the occurrence of EAC from Barrett's esophagus in patients with healthy acid-secreting stomachs.

Why have the prevalence of EAC and esophagogastric junction cancer increased in tandem?

A report has indicated that the American prevalence of EAC and esophagogastric junction cancer have increased in

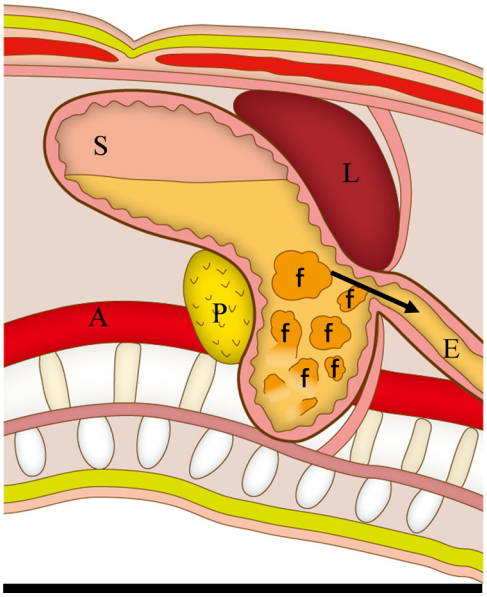

Figure 8 The mechanism of EAC and esophagogastric junction cancer development: A, aorta; L, liver; P, pancreas; S, stomach; f, food remnants. The figure is the schema from the left oblique position of the patient with GERD. The right side of the figure is a head side, and the left side is caudal. If the individual lies down immediately after eating, the food remains in the stomach and collects in the fornix on the dorsal side. The ventral side of the food remnants also includes a component that contains liquid or sludge-like digestive juice, which would also include duodenal fluid including bile acids. The accumulated reflux fluid (arrow) would contain endogenous *N*-nitroso-bile acids, and carcinogenesis could then occur in the esophagogastric junction or esophagus.

tandem.[8] The increasing American prevalence of EAC and esophagogastric junction cancer seem to be related to GERD. Even healthy individuals experience regurgitation of duodenal fluid into the stomach after eating,[120] and the amount regurgitated increases in patients with GERD reportedly. Thus, lying down after eating can result in the duodenal fluid, which contains bile acids, remaining in the stomach (e.g., during sleep).[121] Suzuki *et al*.[122] have reported that the cardia has the highest concentration of nitrates originating from food, which maximizes the luminal generation of *N*-nitroso-compounds from dietary nitrate at the most proximal cardiac region of the acidic stomach. Because the accumulated reflux fluid would contain endogenous *N*-nitroso-bile acids from the stomach, chronic inflammation and carcinogenesis could then occur in the esophagogastric junction or esophagus. Fig. 8 shows this process from the left oblique position, based on a roughly sagittal cross-section that is parallel to the major axis of the esophagogastric junction from the esophagus. The esophagus descends from the oral side to the caudal side along the left side of the aorta, but then proceeds to the right ventral side slightly cranial to the diaphragmatic hiatus, which allows it to pass ventral to the aorta and enter the abdominal cavity near the body's midline, where it transitions into the stomach. If the individual lies down immediately after eating, the food remains in the stomach and collects in the fornix on the dorsal side. When transient lower esophageal sphincter relaxations occur in this state, the esophagogastric junction and lower esophagus would be exposed to these liquids. It is also theoretically possible that the liquids include *N*-nitroso-bile acids, which may play a role in the initiation of carcinogenesis. Thus, the prevalence of EAC and esophagogastric junction cancer has increased in tandem.

CONCLUSION

Barrett's epithelium involves metaplasia of the squamous epithelium into columnar epithelium, which could be described as an adaptation in a microenvironment.[123–125] The microenvironment is decided by mainly bile acids and gastric acid in gastric juice flow backward into the esophagus. Bile acids are known to promote differentiation into intestinal epithelium by increasing the CDX1 and CDX2 expression. Although Barrett's epithelium with intestinal metaplasia involves a higher risk of carcinogenesis than if intestinal metaplasia was absent, cardiac-type mucosa which has the potential to involve intestinal phenotypes can be a risk of EAC. Digestive juice reflux including the high concentration of bile acids induces the production of cytokines, such as NF-κB, has been implicated in carcinogenesis from Barrett's esophagus. This carcinogenic process is likely initiated by the production of *N*-nitroso-bile acids, which is mutagenic and promoted by sustained chronic inflammation.

ACKNOWLEDGMENTS

Ken-ichi Mukaisho has been announced as the winner of The Japanese Society of Pathology; Pathology Research Award in 2017. We are deeply grateful to Prof. Masahiro Ikegami and Dr. Shinichi Hirooka (Department of Pathology, The Jikei University School of Medicine), and Kenichi Goda (Department of Gastroenterology, Dokkyo Medical University), who showed human esophageal adenocarcinoma cases to us for study. We thank Dr. Shizuki Takemura (Department of Diagnostic Pathology, Kusatsu general hospital), who analyzed the German Barrett's adenocarcinoma cases. We also thank Dr. Norihisa Nitta (Department of Radiology, Shiga University of Medical Science), who helped verify the anatomical relationships of the esophagus, esophagogastric junction, and stomach in images from obese patients. Finally, the first author K.M. is deeply grateful to Dr. Suzuko Moritani (Division of Diagnostic Pathology, Shiga University of Medical Science Hospital), who gave him instruction about a pathological diagnosis.

DISCLOSURE STATEMENT

None declared.

AUTHOR CONTRIBUTIONS

KM wrote the paper. SK, RK, TN, TH and HS participated in discussions during the writing of the paper and offered valuable advice.

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