REVIEW

From reflux esophagitis to Barrett's esophagus and esophageal adenocarcinoma

Rui-Hua Wang

Rui-Hua Wang, Department of Gastroenterology, Shanghai Jiao Tong University affiliated Sixth People's Hospital South Campus, Shanghai 201499, China

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Correspondence to: Rui-Hua Wang, MD, Department of Gastroenterology, Shanghai Jiao Tong University affiliated Sixth People's Hospital South Campus, No. 6600 Nanfeng Road, Fengxian District, Shanghai 201499, China. ruihuawang@sina.cn
Telephone: +86-21-57420713
Fax: +86-21-37538586
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Abstract

The occurrence of gastroesophageal reflux disease is common in the human population. Almost all cases of esophageal adenocarcinoma are derived from Barrett's esophagus, which is a complication of esophageal adenocarcinoma precancerous lesions. Chronic exposure of the esophagus to gastroduodenal intestinal fluid is an important determinant factor in the development of Barrett's esophagus. The replacement of normal squamous epithelium with specific columnar epithelium in the lower esophagus induced by the chronic exposure to gastroduodenal fluid could lead to intestinal metaplasia, which is closely associated with the development of esophageal adenocarcinoma. However, the exact mechanism of injury is not completely understood. Various animal models of the developmental mechanisms of disease, and theoretical and clinical effects of drug treatment have been widely used in research. Recently, animal models employed in studies on gastroesophageal reflux injury have allowed significant progress. The advantage of using animal models lies in the ability to accurately control the experimental conditions for better evaluation of results. In this article, various modeling methods are reviewed, with discussion of the major findings on the developmental mechanism of Barrett's esophagus, which should help to develop better prevention and treatment strategies for Barrett's esophagus.

Key words: Animal models; Gastroesophageal reflux disease; Reflux esophagitis; Barrett's esophagus; Esophageal adenocarcinoma

Core tip: Various modeling methods are reviewed and major findings on the developmental mechanism of Barrett's esophagus are discussed, with the aim of identifying better prevention and treatment strategies for Barrett's esophagus. Chronic exposure of the esophagus to gastroduodenal intestinal fluid is an important determinant factor in the development of Barrett's esophagus. However, the exact mechanism of injury is not completely understood. Various animal models have been widely used in research. The advantage of using animal models in research lies in the ability to accurately control the experimental conditions for better evaluation of results.

INTRODUCTION

The occurrence of gastroesophageal reflux disease (GERD) is very common in the human population. The disease has a serious impact on the quality of life[1,2]. Barrett's esophagus is only one known complication derived from GERD. Chronic exposure to gastroesophageal reflux induces the replacement of normal squamous epithelium with specific columnar epithelium in the lower esophagus, leading to intestinal metaplasia, which is closely associated with the development of esophageal adenocarcinoma. Various research models established in studies of the developmental mechanisms of chronic gastroesophageal reflux, esophagitis, intestinal metaplasia, dysplasia, and adenocarcinoma have been widely reported[3].

It is very clear that Barrett's esophagus is the only known form of precancerous lesions of esophageal adenocarcinoma[4,5]. Almost all cases of esophageal adenocarcinoma are derived from Barrett's esophagus. Esophageal adenocarcinoma has a very high degree of malignancy with 5-year of survival of only 15% after diagnosis[6-8]. However, there remain questions as to why some cases of Barrett's esophagus progress into esophageal adenocarcinoma and some do not. The developmental mechanism of the disease is still unclear. However, it is apparent that chronic exposure of the esophagus to gastroduodenal intestinal fluid is an important determinant factor in the development of Barrett's esophagus, although the exact mechanism of injury is not completely understood. There is a need for establishment of stable and reliable research models and strategies for Barrett's esophagus in order to gain a more comprehensive and profound understanding of the developmental mechanism.

Various animal models for disease investigation have been widely used in research, in particular in studies of the developmental mechanisms of disease, and theoretical and clinical effects of drug treatment. The advantage of using animal models in research lies in the ability to accurately control the experimental conditions, allowing better evaluation of the results. Recently, animal models used in research on gastroesophageal reflux injury have made significant progress[9-11]. In this article, various modeling methods are reviewed, with discussion of major findings on the developmental mechanism of Barrett's esophagus, which will allow development of better prevention and treatment strategies for Barrett's esophagus.

REFLUX ESOPHAGITIS

Reflux esophagitis is caused by exposure to gastroduodenal reflux fluid leading to inflammation of the esophagus. Actually, gastroesophageal reflux is a normal physiological process in humans, occurring immediately after a meal. This common phenomenon would not normally cause any damage to the esophageal mucosa, because there is a complete and effective anti-reflux mechanism to protect the esophagus and esophageal mucosa against the damage caused by the entry of reflux fluid. An anti-reflux barrier prevents the reflux entering the esophagus; the esophageal clearance mechanism prevents esophageal reflux fluid staying in the esophagus for too long, and resistance mechanisms protect against esophageal mucosal damage. However, frequent occurrence of gastroesophageal reflux causes inflammation of the esophagus. The etiology and pathogenesis of reflux esophagitis still elicits much controversy; however, the interplay of acid, bile, and a mixture of bile and acid reflux are believed to play an important role in the developmental mechanism.

Studies on the cause of esophagitis and esophageal ulcers had been reported as early as the nineteenth century. In 1839, Albers first described the relationship between inflammation and ulceration of the esophagus[12]. In 1879, Quincke[13] reported three cases of esophageal ulcers that were similar to peptic ulcers, and subsequently described the relationship between acid and pepsin in the development of esophageal epithelial damage. Since acid and pepsin would only damage the esophageal mucosa at pH lower than 2 and such a low pH value was rarely detected in patients with reflux esophagitis, the hypothesis on the role of acid and pepsin in reflux esophagitis was not widely accepted at the time. Only when animal models were successfully established to study the role of acid and pepsin in esophageal mucosal injury was it confirmed that these were important determinant factors in gastroesophageal injury.

In 1938, Selye[14] first conducted pylorus ligation in rats to investigate the effects of acid reflux on esophageal mucosal injury, and confirmed that gastroesophageal reflux esophagitis was highly associated with acid and pepsin. However, one of the disadvantages of using animal models of pylorus ligation was that survival of the animals was greatly shortened by the serious acute injury caused by the surgery, and most did not survive more than 2 d. Wetscher et al[15,16] also used the rat model of pylorus ligation to study reflux esophagitis, and the derived pathological change in reflux esophagitis was found to be different from the pathology of clinical reflux esophagitis. Therefore, the model was found not to be suitable for application to clinical cases of chronic reflux esophagitis. Later, Omura et al[17,18] developed the surgical method of pyloric stenosis in rats, which

was considered to be an animal model of chronic acid reflux esophagitis. The establishment of the animal model of pyloric stenosis laid a technological foundation for the subsequent investigation of pathological changes in acid-induced reflux esophagitis. Barrett[19] developed a technique to recreate the clinical situation of gastroesophageal reflux by longitudinally incising along the lower esophageal sphincter. Another animal model using a surgical approach to create a hiatal hernia led to gastroesophageal reflux. An appropriate surgical procedure would mimic a similar clinical microenvironment for the development of gastroesophageal reflux, which would provide better replication of the clinical microenvironment and would be important for future research into chronic reflux esophagitis.

Acid is not the only component of gastric acid reflux, and duodenal fluid also plays an important role in the pathogenesis of reflux esophagitis. Clinical observations indicated that the incidence of reflux esophagitis in patients with gastrectomy was closely associated with the reflux of duodenal fluid into the esophagus. In 1951, Cross and Wangensteen[20] performed gastrectomies in dogs to produce a back-flow of bile or a mixture of bile and pancreatic juice into the esophagus for the study of the pathological mechanism of reflux esophagitis. Erosion and digestion of the esophageal mucosa caused by exposure to the duodenal fluid was reported.

In 1959, Helsingen[21,22] used animal models created by different surgical methods for the study of reflux esophagitis. Results indicated that the esophageal mucosa would not be damaged without bile in the reflux, and purely a reduction in pancreatic juice in the reflux would prevent damage to the esophagus. However, severe intestinal bile reflux in patents with total gastrectomy and esophagoduodenostomy caused serious damage to the esophageal mucosa leading to partial depigmentation and severe inflammation of the submucosa. An increase in the exposure time to bile reflux increased the damage. Radiology research observed that reflux material gathered in the enlargement of the lower esophagus.

Mud *et al*[23] performed different surgical approaches in an animal model to allow gastric acid, bile and pancreatic juice, separately or jointly entering into the esophagus in order to investigate the mechanisms of esophageal mucosal injury, and he believed that the duodenal fluid was critical for the development of esophageal mucosal inflammation. An animal model using direct perfusion of different components of duodenal reflux material into the esophagus could help to determine their role in the development of disease, and also allowed accurate control of experimental conditions, and the amount of bile and acids in each exposure and other determinant factors. Continuous perfusion of reflux materials into the esophagus successfully induced ulcers and severe esophagitis in a rat model of esophageal perfusion. However, this model was believed to be more reliable in producing the condition of chronic reflux esophagitis, and could

not induce the conditions of Barrett's esophagus and esophageal adenocarcinoma over 7 d and 4 wk, respectively[24-27]. The advantage of using an esophageal perfusion model is the use of small animals and the avoidance of complications related to malnutrition. The mortality rate of animals is low, with a high long-term survival rate, and the experimental conditions could be well controlled, thus reducing the time required to induce esophageal injury in the animals.

EXPERIMENTAL COLUMNAR EPITHELIUM OF THE ESOPHAGUS

In 1950s, the British scientist Barrett[28] firstly described the pathological phenomenon of red columnar epithelium in the lower esophageal mucosa. However, the etiology, pathophysiology and clinical treatment was disputed in the medical community for some time. Different terminologies, including gastric mucosal epithelium and congenital short esophagus had been used to describe the lower esophageal columnar epithelium. Later, the name was established as Barrett's esophagus. The prevailing theory about the origin of Barrett's esophagus was initially thought to be congenital, and derived from the time of embryonic development[28]. The supporting evidence for the hypothesis of innate factors included the observation of isolated metaplasia of the intestinal epithelium in the upper part of the esophagus, and the occurrence of Barrett's esophagus in babies and children[29].

Barrett's esophagus involves metaplasia of the abnormal columnar epithelium of the esophagus, replacing the stratified squamous epithelium in the lower esophagus. The pathology of Barrett's esophagus could be divided into three different types: 1, specific intestinal epithelium with goblet cells; 2, epithelium without goblet cells at the junction of the gastric cardia and pylorus; 3, gastric mucus or acid-secreting epithelium without goblet cells[30]. The diagnosis of Barrett's esophagus was controversial, with a dispute on whether the diagnosis should be based on the presence of columnar epithelium or intestinal histologic findings. The American Gastroenterological Association in Chicago[31] had set the diagnostic criteria for Barrett's esophagus based on the presence of intestinal metaplasia at the squamous columnar cell junctions and gastroesophageal junction. However, the British Society of Gastroenterology did not consider the above diagnostic criteria to be the necessary conditions for the diagnosis of Barrett's esophagus.

Immunohistochemistry confirmed that the ectopic columnar epithelium was different from both the embryonic characteristics of the gastric mucosa and that of Barrett's esophagus[32]. Allison and Johnstone[33] in 1953 described the ulcers in the columnar epithelium as "Barrett ulcers" which were histologically confirmed to contain both goblet cells and villous enterocytes. As all patients with Barrett's esophagus have symptoms of

gastroesophageal reflux and hiatal hernia, the authors believed that metaplasia of the intestinal epithelium was a chronic gastroesophageal complication derived from reflux esophagitis. The hypothesis that Barrett's esophagus was acquired rather than congenital was later gradually supported by various scientific institutes and communities. Research suggested that the specific type of columnar epithelium was different from the normal epithelium at the gastric cardia, but it was similar to that found in the intestinal metaplasia of patients with total gastrectomy and esophagogastric anastomosis[34-38]. A number of studies on the relationship between the special columnar epithelium and gastric mucosa were reported[39-41]. Clinical studies also showed that the columnar epithelium appeared secondary to gastroesophageal reflux caused by the barrier dysfunction of the gastroesophageal sphincter, leading to back-flow of the gastric fluid into the esophagus.

Owing to the lack of animal model-based experiments, there was much speculation about the origin of intestinal metaplasia cells during the 1950s and 1960s. In 1968, Hennessey spent 4 wk attempting to induce metaplasia of the intestinal epithelium in dogs, but without success[42]. The first canine model of Barrett's esophagus was later successfully established, and was used to confirm the acquired nature of the pathology of Barrett's esophagus[10].

In 1970, Bremner successfully established a canine model of Barrett's esophagus for the study of metaplasia of the columnar epithelium lining the lower esophagus. The method used a surgical approach to induce gastrointestinal fluid back-flow into the esophagus to damage the normal esophageal mucosa. The model was divided into three groups. Group 1 used surgical means to peel off the mucosa in the lower esophagus, and the longitudinal muscle was surgically incised to form a hiatal hernia leading to the induction of gastroesophageal reflux. Group 2 had the same operation with additional application of histamine to stimulate gastric acid secretion. Group 3 acted as the control. Results showed that the visible portion of the metaplasia of the columnar epithelium was found to partly or completely cover the lower esophagus, and the extent of metaplasia was closely associated with the degree of gastroesophageal reflux. Under conditions of gastroesophageal reflux, the area lacking squamous cells regenerated, with new cells that almost completely became columnar epithelium. The regenerated columnar epithelium did not extend upward from the gastric mucosa, but was closely associated with reflux esophagitis. Experimental results suggested that the columnar epithelium lining the lower esophagus was caused by damage to the squamous epithelium leading to the upward migration of columnar epithelium from the stomach or gastroesophageal junction epithelium. The study confirmed that intestinal metaplasia at the lower esophagus occurred via damage to the esophageal mucosa, dysfunction of the lower

esophageal sphincter, a reduction in acid clearance, and an increase in acid reflux. The study also showed that it would take at least take 8 wk to form the mucosal metaplasia. It became the landmark study to show the acquired nature of the pathological state of columnar epithelium lining the lower esophagus. Bremner's view on the derivative nature of the columnar epithelium that extended in the direction of the esophagus from the gastric epithelium was later considered to be wrong by the research society.

Other studies also confirmed that the intestinal metaplasia in the lower esophagus was acquired. Researchers believed that these cells originated from the esophageal submucosa. Many research techniques had to use the surgical procedure of mucosal dissection in order to induce the formation of metaplasia, while other surgical approaches could only induce esophagitis. In 1983, Pollara et al[43] reported that surgical resection of the squamous epithelium induced metaplasia of the columnar epithelium lining the lower esophagus. The method included the dissection of the squamous mucosa and the destruction of the function of the lower esophageal sphincter. The metaplastic columnar epithelium was found to turn into small glands and villous-like small intestinal mucosal cells. The severity of metaplasia significantly increased with prolongation of the study period. Adler[44] also reported similar results, but did not discuss the cell origin of the metaplasia.

In order to clarify the origin of metaplastic intestinal epithelial cells and the impact of GERD on metaplasia, Gillen et al[45] established a canine model of esophageal metaplasia in 1988. After stripping off the bridge-like squamous epithelium, reflux of bile and a mixture of gastric acid and bile was induced. Results indicated that only reflux of the mixture of gastric acid and bile, and not bile reflux, could induce metaplasia of the columnar mucosa at the stripped zone of the squamous mucosa in the lower esophagus. The study clearly indicated that the metaplasia developed from the upward migration of gastric mucosa. The epithelial metaplasia was derived from the villous goblet cells, parietal cells, and false absorptive cells. The authors speculated that the origin of these cells might be the pluripotent stem cells of esophageal glands. Both gastrointestinal morphology and mucous histochemical staining indicated that the origin of the cells was different from that of the gastric cardiac epithelium. Gillen had used surgery in the canine model to remove the squamous epithelium barrier at the lower esophagus to trigger reflux of the mixture of gastric acid and bile to create the metaplasia. It was found that metaplasia of the glandular epithelium could cross the squamous epithelial barrier, and be present at the area of mucosal injury. The authors speculated that epithelial metaplasia originated from the differentiation of pluripotent stem cells of the esophageal mucous glands, but not the gastric epithelium.

In order to study the effects of the local micro-environment on lower esophageal mucosal damage,

Li et al[46] used the canine model to strip the mucosa at the lower esophagus to induce gastroesophageal reflux. Studies have shown that the repair mode for mucosal damage was associated with the degree of esophageal mucosal damage. If the squamous epithelium was damaged, it would then be replaced with cells mixed with squamous epithelial cells. However, if the submucosal duct cells were damaged, only columnar epithelial cells would be produced in the repair process. It had already been confirmed that metaplastic columnar epithelium was similar to the intestinal metaplasia of Barrett's esophagus epithelium. The model of mucosal damage induced by a surgical approach was similar to human esophageal injury; however, it differed from clinical esophagitis caused by esophageal reflux which leads to esophageal ulcers.

Surgery in animal models to create esophageal anastomosis has been widely used to study reflux esophagitis, but did not produce Barrett's esophagus in earlier studies. In 1996, Miwa et al[47] conducted five types of surgical procedures for esophageal anastomosis, and total gastrectomy with esophageal anastomosis in rats to produce gastric and duodenal content reflux, duodenal content reflux only, gastric acid reflux only, and other reflux in order to investigate the role of reflux esophagitis in columnar metaplasia lining the lower esophagus. The authors believed that the duodenal content played an important role in the occurrence of Barrett's esophagus. Another surgical approach using total gastrectomy with esophageal anastomosis further confirmed the above result. Seto et al[48] used the method of total gastrectomy with esophageal anastomosis and acidic liquid infusion to induce the development of columnar epithelium in the esophagus. Goldstein used a rat model of esophagoduodenostomy with an added iron diet to significantly increase the incidence of metaplasia of the esophageal intestinal epithelium[49,50]. Wong et al[51] studied esophageal mucosal damage in rats caused by surgery, and showed that damage repair of esophageal mucosal injury led to metaplasia of the lower esophagus. Seto and Kobori et al[48] used an animal model of total gastrectomy with esophageal gastrointestinal anastomosis surgery and acidic liquid perfusion to demonstrate the role of reflux esophagitis in promoting metaplasia of the intestinal epithelium, and acid further exacerbated the pathological phenomenon. Columnar epithelium was found to survive better than squamous epithelium in an acidic environment. Wong and Finckh's[51] studies suggested that the migrated epithelium could not form columnar epithelium at the distal end of the esophagus, as the absence of basement membrane was an essential condition for the formation of columnar epithelium. Deep mucosal damage to the submucosa caused by chronic gastroesophageal reflux could destroy mesenchymal cells, and trigger the upward migration of basal cells to initiate the nascent development of columnar epithelium.

Columnar epithelium developed into its original state that became more tolerant than normal tissue against the attack of various damaging factors. It was suggested that the healing mechanism would allow the esophagus to better adapt and protect itself from further damage by acid and bile.

With the use of surgical procedures in animal models to create chronic reflux esophagitis and intestinal metaplasia, the pathophysiology was more in line with that of human disease. At present, these surgical procedures have been improved with gastrectomy and gastric antrum resection, to induce better back-flow of duodenal fluid and/or gastric fluid into the esophagus. These animal models became widely adopted for the study of the mechanism of Barrett's esophagus[52-59].

EXPERIMENTAL ESOPHAGEAL ADENOCARCINOMA

In 1952, Morson and Belcher[60] reported a case of adenocarcinoma of the columnar epithelium in the esophagus. Subsequent studies confirmed the correlation of esophageal adenocarcinoma and Barrett's esophagus[61]. In the United States and other developed countries, the incidence of esophageal adenocarcinoma has increased significantly[62]. In the past few decades, the epidemiology of esophageal cancer has markedly changed. The incidence of esophageal adenocarcinoma is rapidly increasing, while that of esophageal squamous cell carcinoma has gradually decreased. The change indicates that differences exist in the pathogenesis of the two tumors, and the carcinogenic environment has been altered. The vast majority of esophageal adenocarcinoma was found to originate from Barrett's esophagus. Experimental animal model studies further supported the theory of the close relationship between esophageal reflux and esophageal adenocarcinoma.

Specific nitrosamines and other carcinogens could affect the esophageal epithelium and induce esophageal squamous cell carcinoma in rats[63,64], but these compounds could not lead to esophageal adenocarcinoma. Although an animal model of esophageal adenocarcinoma had been studied a long time ago, it was not successful, possibly because an incorrect surgical method was used and the time for induction was too short. In 1989, Pera et al[52] successfully induced esophageal adenocarcinoma in rat using surgery and application of a carcinogen. Subsequently, Attwood used the animal model with carcinogens to study the occurrence and development of esophageal adenocarcinoma, and concluded that only the application of gastric acid and carcinogens would not induced the development of esophageal adeno-carcinoma. Only duodenal reflux occurring in animals with an intact stomach or animals with gastrectomy and esophagoduodenostomy developed esophageal

adenocarcinoma.

There are many reports describing several surgical models for induction of esophageal adenocarcinoma. Miwa performed duodenal anastomosis in rats to induce duodenal gastroesophageal reflux leading to a 17% incidence of esophageal adenocarcinoma[65]. Mirvish *et al*[53,54] also performed duodenal anastomosis in rats to induce rates of esophageal adenocarcinoma of 42% and 33%, respectively. Xu *et al*[66] performed total gastrectomy with esophagojejunostomy in rats, resulting in esophageal adenocarcinoma in 12.2%. The development of esophageal adenocarcinoma occurred at around 3-6 mo after surgery[67]. The tumor was located at the lower esophageal anastomosis or at the mouth of the anastomosis within the top few centimeters. The tumor was an esophageal adenocarcinoma or mixed form of squamous carcinoma, but no case of squamous cell carcinoma alone was reported.

Studies in animal models showed that proton pump inhibitors such as omeprazole induced duodenal gastroesophageal reflux leading to esophageal mucosal hyperplasia[68], and excessive use of acid inhibitors enhanced the alkaline duodenal content reflux, leading to development of Barrett's esophagus and esophageal adenocarcinoma. The presence of gastric acid protected against duodenal reflux to the esophagus. Studies showed that gastric duodenal esophageal reflux was more harmful than gastric esophageal reflux, which played an important role in Barrett's esophagus and esophageal adenocarcinoma[69].

Currently, surgery-induced animal models of esophageal adenocarcinoma have been affirmed, and studies have shown a sequence from reflux esophagitis, Barrett esophagus, and dysplasia to esophageal adenocarcinoma. Studies also showed that gastroesophageal reflux could enhance the role of carcinogens, and excess iron load and a high-fat diet also played an important role in the development of esophageal adenocarcinoma[59]. However, controversy still exists, with contradictions in the time of occurrence and development of esophageal adenocarcinoma. The developmental mechanism of carcinogen-induced esophageal adenocarcinoma in experimental studies was found to be different from that of clinical esophageal adenocarcinoma.

SURGICAL PROCEDURES IN ANIMAL MODELS

Surgical approaches for induction of esophageal injury in animal models included: (1) pylorus ligation; (2) esophagogastroplasty; (3) esophageal perfusion; (4) intact stomach or gastrectomy with esophageal anastomosis; (5) esophagojejunostomy; and (6) esophagus mucosal stripping with hiatal hernia plasty.

Pylorus ligation
Selye[14] used the method of pylorus ligation to study the damaging effects of gastroesophageal reflux in the esophagus of rats in 1938. Wetscher *et al*[15,16] ligated the duodenum to generate reflux esophagitis and studied the role of oxygen free radicals in the esophagus. However, the surgical procedure only allowed the rats to survive for 1-2 d to produce the condition of acute reflux esophagitis, and was not suitable for the study of chronic reflux esophagitis.

Esophagogastroplasty
The method used a surgical procedure to damage the esophageal sphincter in order to induce gastro-esophageal reflux. Acid reflux alone could only induce chronic reflux esophagitis, but not Barrett's esophagus and esophageal adenocarcinoma[19,42,70,71].

Esophageal perfusion
This method involved a catheter or pre-buried micro-pump in the body of the animal to continuously infuse acid, bile, or pancreatic juice into the esophagus to study the effect of different components of reflux material on the esophageal mucosa. The method allowed the accurate control of experimental conditions and factors for the induction of ulcers and severe esophagitis. The advantage of the method was that the induction time for esophageal injury was short, and was suitable for the study of chronic reflux esophagitis, but not Barrett's esophagus and esophageal adenocarcinoma[24-27].

Esophagojejunostomy
Esophagojejunostomy involves preservation of the stomach or removing the stomach at the lower end of the esophagus and above the pylorus followed by connecting the lower end of esophagus with the jejunum to induce jejunal esophageal reflux. As the reflux material contained duodenal content, it could induce Barrett's esophagus and esophageal adenocarcinoma. The experiment determined an important role of duodenal fluid in the development of Barrett's esophagus[47,55-57].

Jejunal esophagogastric anastomosis
This method connected the jejunum to the esopha-gogastric anastomosis in rats to induce reflux of gastric acid and duodenal fluid into the esophagus, creating a condition similar to that of human esophageal reflux. Barrett's esophagus developed after 20 wk, while esophageal adenocarcinoma appeared after 40 wk[72].

Esophageal hiatal hernia plasty
The lower esophageal squamous mucosa or the bridge-like squamous mucosa was stripped to induce hiatal hernia and the reflux of duodenal fluid. This method provided powerful evidence to show the cell origin of Barrett's esophagus was not derived from migration of the gastric mucosa to the esophagus, but instead, originated from differentiation of pluripotent stem cells of esophageal glands[10,45].

DIFFERENT TYPES OF ANIMAL MODELS

In the study of esophageal reflux mucosal injury, the phenomenon of reflux involves dysfunction of the lower esophageal sphincter, a lack of esophageal motility, damage to the esophageal mucosa, abnormal metaplasia, precancerous lesions, and carcinogenesis, which are difficult to investigate in humans. Different animal models are required for in-depth investigations.

Rat model

Compared with a large-animal model, the advantage of rats is that this animal model is economical, reproducible, and experimental conditions are easily controlled. The role of gastric acid and pepsin in esophageal injury were well delineated using animal models of acute reflux esophagitis and chronic reflux esophagitis[15-18]. The establishment of an animal model producing reflux of a mixture of gastric and duodenal fluid successfully induced metaplasia of the columnar epithelium at the lower esophagus and esophageal adenocarcinoma. The establishment of a rat model of reflux esophagitis from theory to practice provided various evidences to show the mechanisms in the development of esophageal adenocarcinoma[49,50].

Rabbit model

The rabbit model used a micro-pump to continuously infuse different components of gastric and duodenal fluid into the esophagus, and was useful in the study of acid and protease-induced reflux esophagitis to determine the roles of nitric oxide and growth factor receptor in epidermal esophageal mucosal damage[24,73-76]. Studies showed that the pH of the reflux material played a more important role than proteases in damaging the esophageal mucosa, such that the protease could induce injury only at the critical point of acid load at pH < 3[77]. Excessive and prolonged bile reflux leading to the accumulation of a mixture of bile and acid near the mucosal cells, and the unconjugated bile salt could cause more damage to the esophageal mucosal barrier in an acidified low pH environment[78,79] than in alkaline conditions[80-83].

Canine model

This model has made a major contribution to the study of the cell origin and the developmental mechanisms of esophageal metaplasia. In 1970, Bremner et al[10] first proved that the lower esophageal columnar epithelium was acquired. Gillen et al[45] established a canine model of esophageal metaplasia in 1988, and proved that metaplasia of the esophageal epithelium was derived from pluripotent stem cells of the esophageal gland, but not from migration of the gastric mucosal epithelium.

Porcine model

This model had structural and physiological features similar to that the humans. Its low cost and wide practicality made it one of the most promising animal models in research studies. The various anesthetic and surgical procedures used were also similar to that for humans. The size of the animals was comparable to that of humans making surgery easy, and provided practical features in studies of the function of the gastroesophageal sphincter. Rat and rabbit species are relatively far removed from humans, and the size of the animals was relatively small compared with that of pigs, making the pig more suitable for the study of certain human diseases, especially gastroesophageal pathophysiology. Research using the porcine model to study the clearance of esophageal acid reflux and the impact of reflux on esophageal mucosal damage[84], could help in the development of new anti-reflux therapy[85,86], and exploration of new mechanism of anti-reflux drugs[87].

Transgenic animal model

The transgenic animal model can be used to study the function of specific genes, and enhance the investigation of the disease pathogenesis. Transgenic or knockout mice are animal models commonly used for the study of disease pathogenesis. A study using K14-Cdx2 transgenic mice showed that basal stem cells of the esophagus can express Cdx2, and Cdx2 ectopic expression could alter the cellular phenotype, cell barrier, and differentiation[88]. The changes were found to be in a transitional state of the normal squamous epithelium and columnar epithelium. K14-Cdx2 mice represented a useful model to study the progression from squamous epithelium to Barrett's esophagus. p63-deficient mice that lacked squamous epithelia may be used as a model of acid-reflux damage. One study showed that p63 null embryos rapidly developed intestine-like metaplasia with gene expression profiles similar to that of Barrett's metaplasia, which suggested that Barrett's esophagus may be initiated from opportunistic competitive interactions between cell lineages, rather than genetic alterations[89]. Previous studies had shown[90] that p27-knockout mice with esophageal anastomosis combined gastrectomy surgery had a significantly higher incidence of Barrett's esophagus and esophageal adenocarcinoma.

Pigs are often used in the surgical training of surgeons to improve their proficiency and accuracy in surgery[91]. Genetic modification technology in pigs has made significant progress in recent years. Nowak-Imialek et al[92] established the OG2 transgenic pig model, which could induce pluripotent stem cells. It was a new method for the study of pluripotent stem cells in pigs. Relevant studies have already screened out the highly sensitive, reproducible platform of pig DNA microarrays[93]. It is foreseeable that the transgenic pig model or transgenic pig model combined with anti-reflux surgery will be used in the near future to study the pathogenesis and intervention

strategies of Barrett's esophagus and esophageal adenocarcinoma. Although digestive tract anatomy and physiology in higher mammals is very similar to that of humans, different animal models were found to be suitable for the study of different aspects of diseases[94]. Very often, several different animal models are required for the study of same disease, and could help to provide different views on the understanding of disease, with a view to prevention and treatment.

CONCLUSION

The establishment of animal models could provide a technological platform for the research of esophageal reflux, which would allow a deeper understanding of the developmental mechanisms of esophageal reflux, reflux esophagitis, Barrett's esophagus, and esophageal adenocarcinoma. The investigation of the developmental mechanism of Barrett's esophagus and esophageal adenocarcinoma caused by surgery-induced reflux damage was very important to lay a solid theoretical foundation for the prevention and clinical treatment of precancerous lesions and esophageal adenocarcinoma.

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