Medical Sciences

Current Animal Models of Gastroesophageal Reflux Disease, Barrett’s Esophagus, and Esophageal Adenocarcinoma

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The incidence of esophageal adenocarcinoma (EAC) is rapidly increasing in the United States and is becoming a serious problem. This disease is known to develop through a metaplasia–dysplasia–carcinoma sequence. Gastroesophageal reflux disease (GERD) is the most common risk factor in the progression of inflammation—Barrett’s Esophagus (BE)—EAC. Although an individual’s risk of progression is quite low, the near-pandemic prevalence of GERD makes it an important health concern. Unfortunately, by the time patients with EAC are symptomatic, they are generally in a later stage of the disease, which increases the likelihood of a poor prognosis. Thus, early clinical diagnosis is critically important. To improve management of patients with EAC, the molecular pathophysiology of the disease must be elucidated. Animal models have potential to provide answers, and the primary tools that can provide further understanding of the mechanism of GERD–BE–EAC progression.

Animal model | Barrett’s esophagus | esophageal adenocarcinoma | gastroesophageal reflux disease | GERD

Introduction

The incidence of esophageal adenocarcinoma (EAC) is rapidly increasing in the United States, in contrast with the declining trends seen in most other cancer types. An estimated 16,910 new cases of esophageal cancer are diagnosed each year, and the histological subtype EAC accounts for more than 60% of these cases.

EAC is thought to develop in a sequential manner: through a metaplasia–dysplasia–carcinoma sequence. Chronic inflammation of the distal esophagus that results from gastroesophageal reflux disease (GERD) often leads to Barrett’s esophagus (BE). In patients with BE, the metaplastic condition in which normal squamous epithelium is replaced by intestinal columnar epithelium occurs. BE sometimes progresses to low-grade dysplasia, high-grade dysplasia, and finally adenocarcinoma.

GERD is firmly established as the primary risk factor for BE and EAC, but other variables contribute to progression of GERD (e.g., obesity, male sex, age, and tobacco). Previous studies have demonstrated that chronic reflux of mixed gastric acid and bile is linked to the pathogenesis of BE and EAC, and the risk of EAC is reportedly much as 30 times higher in patients with BE than in members of the general population. However, it is extremely difficult to investigate the natural history of EAC progression in humans, as just 0.4% of cases of BE develop to EAC each year. Therefore, animal models are great potential tools that can provide further understanding of the mechanism of GERD–BE–EAC progression.

Various animal models used to study GERD, BE, and EAC have included rats, mice, rabbits, dogs, and baboons. Rodents, especially rats and mice, are particularly well-suited to laboratory analysis given their small size, relative low cost of maintenance, and widespread availability. Furthermore, they have a stratified squamous epithelium-lined esophagus similar to the human esophagus. However, several histological differences exist between the rodent and human esophagus, such as the presence of keratinized epithelium and the absence of submucosal glands in rodents.

Until recently, rats were the primary animal used to study GERD, BE, and EAC models. After complete mapping of the mouse genome, we have three basic options of animal models that are natural, surgical, and genetic models. Several genetic modified mouse models with or without surgical facilitation have recently been reported with good success. In the sections that follow, we review the development of GERD, BE, and EAC models in rats and in mice.

Animal Models

Rat

Rodents, including rats and mice, do not have spontaneous reflux. In these animals, then, reflux must be surgically induced. Surgical models can be categorized into four subtypes: 1) only gastric secretion reflux (GER), 2) only duodenal secretion reflux (DgER), 3) duodenogastric reflux with “bile predominance” (DgER), and 4) duodenogastric reflux with “acid predominance” (dGER).

Rat GERD Models

In 1938, Selye16 reported the first animal reflux model in which GER was induced by ligating the gastric pylorus in rats (Fig. 1A). This model showed hemorrhagic esophagitis or esophageal perforation within 17 hours of the operation. However, this model could not be studied beyond the first 24 hours due to this acute fatal esophageal injury (which was dissimilar to human reflux).

Sixty years later, Omura et al.17 described a chronic acid reflux model that created a sufficient observation period that was more applicable to humans. They induced chronic esophagitis by both gastric volume reduction (i.e., ligating the limiting ridge between the forestomach and the glandular portion) and partial gastric outlet obstruction (i.e., covering the pyloric ring with a small piece of an 18 Fr Nélaton catheter) (Fig. 1B). This model is now well established as surgically induced chronic GERD, and is commonly used in a wide range of study fields, including the evaluation of the pathophysiology and the investigation of drug efficacy. Interestingly, GER models do not progress to BE or EAC even with the addition of exogenous carcinogens, although squamous cell carcinoma has been shown to be induced in these models.

Conflict of interest: No conflicts declared.

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Figure 1. Surgical reflux models. (A) Acute acid reflux model; GER.16 (B) Chronic acid reflux model; GER.17 (C) Esophagojejunalostomy; DgER.5,22 (D) Esophagoduodenostomy (end-to-side anastomosis); DgER.21 (E) Esophagoduodenalostomy (side-to-side anastomosis); DgER.7 (F) Esophagojunostomy with gastrectomy; DER.23 (G) Esophagogastroduodenal anastomosis; dGER.24
Table 1. Animal models and resultant esophageal adenocarcinoma.

<table>
<thead>
<tr>
<th>Study, year</th>
<th>Animal</th>
<th>Genetic modification</th>
<th>Surgical procedure</th>
<th>Reflux type</th>
<th>Carcinogen used</th>
<th>EAC incidence</th>
<th>Study duration (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pera, 1989&lt;sup&gt;22&lt;/sup&gt;</td>
<td>Rats</td>
<td>None</td>
<td>Esophagojejunostomy</td>
<td>DgER</td>
<td>DMNM</td>
<td>38%</td>
<td>19 (average)</td>
</tr>
<tr>
<td>Atwood, 1992&lt;sup&gt;9&lt;/sup&gt;</td>
<td>Rats</td>
<td>None</td>
<td>Esophagoduodenostomy</td>
<td>DgER</td>
<td>DMNM</td>
<td>35%</td>
<td>22</td>
</tr>
<tr>
<td>Miwa, 1996&lt;sup&gt;7&lt;/sup&gt;</td>
<td>Rats</td>
<td>None</td>
<td>Esophagojejunostomy</td>
<td>DgER</td>
<td>None</td>
<td>75%</td>
<td>50</td>
</tr>
<tr>
<td>Chen, 1999&lt;sup&gt;25&lt;/sup&gt;</td>
<td>Rats</td>
<td>None</td>
<td>Esophago-gastro-duodenal anastomosis</td>
<td>DER</td>
<td>None</td>
<td>54%</td>
<td>40</td>
</tr>
<tr>
<td>Fein, 1999&lt;sup&gt;27&lt;/sup&gt;</td>
<td>Mice</td>
<td>p53 knockout</td>
<td>Esophagojejunostomy with gastrectomy</td>
<td>DER</td>
<td>None</td>
<td>50%</td>
<td>24</td>
</tr>
<tr>
<td>Ellis, 2001&lt;sup&gt;29&lt;/sup&gt;</td>
<td>Mice</td>
<td>p27 knockout</td>
<td>Esophagojejunostomy</td>
<td>DgER</td>
<td>MBN</td>
<td>6%</td>
<td>18-20</td>
</tr>
<tr>
<td>Lechpammer, 2005&lt;sup&gt;30&lt;/sup&gt;</td>
<td>Mice</td>
<td>p27 knockout</td>
<td>Esophagojejunostomy</td>
<td>DgER</td>
<td>MBN</td>
<td>32%</td>
<td>20</td>
</tr>
<tr>
<td>Quante, 2012&lt;sup&gt;31&lt;/sup&gt;</td>
<td>Mice</td>
<td>L2-IL-18</td>
<td>None</td>
<td>N/A</td>
<td>N/A</td>
<td>22.2%</td>
<td>86-94</td>
</tr>
</tbody>
</table>

Abbreviations: DER, only duodenal secretion reflux; DgER, duodenogastric reflux with “bile predominance”; dGER, duodenogastric reflux with “acid predominance”; DMNM, 2,6-dimethylnitrosomorpholine; EAC, esophageal adenocarcinoma; MBN, N-methyl-N-benzyl nitrosamine; MNAM, methyl-n-amylnitrosamine.

Rat BE–EAC Models
In 1989, Pera et al.<sup>22</sup> reported EAC induction in rats that underwent esophagojejunostomy (DgER) (Fig. 1C) with a weekly injection of the carcinogen 2,6-dimethylnitrosomorpholine (DMNM). They found an incidence of EAC of 38%, and its average survival time was 19 weeks. No EAC was observed in rats who underwent weekly injections of DMNM without surgery, although squamous cell carcinoma was noted. BE was observed in this surgical reflux model whether the rats were exposed to 2,6-DMMN or not.

Atwood et al.<sup>9</sup> confirmed these findings in 1992, using rats that underwent esophagoduodenostomy (DgER) (Fig. 1D, E) and were treated with carcinogens (either DMNM or methyl-n-amylnitrosamine [MNAM]). This model showed an incidence of EAC similar to that reported by Pera et al., finding 35% EAC 22 weeks postoperatively. The most noteworthy finding from this study was that one rat, the DgER model, showed EAC without any carcinogen introduced. In 1996, Miwa et al. found the same thing.<sup>7</sup> They showed that DgER models (esophagojejunostomy and DER models (esophagojejunostomy with gastrectomy) (Fig. 1F) had high incidences of EAC (75% and 54%, respectively) 50 weeks after surgery even in the absence of exogenous carcinogens. Chen et al.<sup>25</sup> suggested that the esophagogastrroduodenal anastomosis (dGER) (Fig. 1G) model may be best suited for this type of study, as it avoids nutritional deficiency and severe diffuse esophagitis.

The effect of nutritional status on EAC development was also investigated in several studies. Carcinogenic process was found to possibly be promoted by a diet high in fat<sup>24,25</sup> and iron supplementation<sup>26</sup> after surgical induction of reflux in rats. Reflux models and their associated EAC incidence rates are summarized in Table 1.

Mouse
Surgical reflux models in mice are more technically challenging than in rats due to their smaller size and relative intolerance to surgical stress. However, mouse reflux models have the advantage of available genetic modifications, which are especially useful for oncogenetic investigations. Therefore, transgenic mouse models with specific knockout genes are commonly used as BE–EAC models.

Mouse BE–EAC Models
In 1999, Fein et al.<sup>27</sup> reported the first genetic mouse model with surgical facilitation. They used p53 knockout mice and carried out esophagojejunostomy with gastrectomy (Fig. 1F) (DER). This model showed a high rate (i.e., 50%) of EAC development. However, only 4 of 12 mice survived after 24 weeks of observation, which made it a poor model.<sup>28</sup> Ellis et al.<sup>29</sup> and Lechpammer et al.<sup>30</sup> reported an EAC model using p27 knockout mice that underwent esophagojejunostomy (DgER) (Fig. 1C) and were also treated with the carcinogen N-methyl-N-benzyl nitrosamine (MBN). This model showed 100% survival during the study period, although the incidence of EAC was relatively lower (20%-32%) after 18–20 weeks of observation.

In 2012, Quante et al.<sup>31</sup> succeeded in creating a transgenic model that showed spontaneous development of esophageal adenocarcinoma without any surgical facilitation or carcinogen. They overexpressed human interleukin-1p in the esophagus and squamous forestomach mucosa in mice using Epstein-Barr virus promoter. In this model, esophagitis was seen in all mice by 6 months of age, BE developed in 90% mice at 12-15 months, and 22.2% of mice developed high-grade dysplasia or EAC at 20-22 months. Furthermore, the BE–EAC development was accelerated by bile acid oral intake.

Limitations of Current Animal Models
Although various reflux models have been described, it is still difficult to translate these results directly from animal models to human disease. A review by Hackam et al.<sup>32</sup> showed that highly cited animal studies rarely translate into successful human research. They reported that 45% of the animal studies remain untested, 18% were contradicted, and only 37% were replicated in human research. This is because several differences exist in the histological structures and the expression profiles of the molecular markers between humans and animals.<sup>33</sup> Additionally, EAC in animal models can neither invade locally nor metastasize.<sup>34</sup> This finding prompts the question: Are the tumors
induced in animals different from human adenocarcinomas, even though they resemble human EAC histologically?

Furthermore, we don’t have answers about whether the results of animal studies can have universality even at the animal level. Yano et al.\textsuperscript{35} showed significant differences in growth curves and eating patterns in the same species found on different continents.

This potential gap limits the universal applicability of reproducing animal models developed in a particular region.

The problems delineated above remain as the future issues to be resolved in current animal models. A more appropriate model is required if we wish to extrapolate our findings to be relevant in human disease.

6. Attwood SE, Smyrk TC, DeMeester TR, Mirvish SS, Stein HJ, Yano et al.\textsuperscript{35} showed significant differences in growth curves and eating patterns in the same species found on different continents.