A Mouse Model for Barrett’s Esophagus: Surgery and Histology

Taddei A*, Lottini T, Fazi M, Ringressi MN, Lasraioili E, Bechi P and Arcangeli A

1Department of Surgery and Translational Medicine, University of Florence, Italy
2Department of Experimental and Clinical Medicine, Section of Internal Medicine, University of Florence, Italy

Abstract

Purpose: Barrett’s esophagus (BE) is the sole precursor lesion of esophageal adenocarcinoma (EA) identified so far. The progression towards EA is estimated to affect 2 to 10% of BE patients, hence endoscopic surveillance of at-risk subjects is mandatory. Surveillance endoscopic procedures imply high cost, discomfort and risks for the patient, as well as the non-infrequent missing of small, focal lesions signaling progression to EA. Hence, it is important to search for new potential markers to better identify BE patients at risk of EA progression. The aim of this study was to produce a mouse model of BE, suitable for further molecular and genetic analyses.

Methods: Forty-four CD1 mice were operated upon by means of an esophago-jejunal anastomosis. Five CD1 mice underwent a sham operation. The animals were sacrificed 10 months later and histological analysis was performed with Hematoxylin & Eosin and Alcian Blue staining.

Results: The overall postoperative mortality rate was 11%. Of the 39 operated animals 14% developed histologically detectable intestinal metaplasia in the lower esophagus. No histologically detectable lesions were shown in the sham group.

Conclusions: The mice model we propose could be applied because of its technical feasibility and acceptable mortality and can be used in transgenic mice too, in order to better understand molecular progression from BE to esophageal adenocarcinoma.

Keywords: Barrett’s esophagus; Mouse model; Intestinal metaplasia; Histology

Introduction

Barrett’s esophagus (BE) is a condition where the normal squamous epithelium of the esophagus is replaced by intestinal-type columnar epithelium. BE diagnosis is based on the presence of goblet cells of the intestinal type within columnar epithelium [1]. BE is thought to be a precursor lesion for esophageal adenocarcinoma (EA). Despite the relatively low progression rate of BE towards EA (EA is estimated to affect 2 to 10% of BE patients), endoscopic surveillance of at-risk subjects is commonly applied. However, routine endoscopic procedures must be applied with caution due to their cost, discomfort and risks for the patient, as well as the non-infrequent missing of small, focal lesions signaling progression to EA. Considering that BE incidence has been sharply increasing in the last decades in Western countries [2] and that prognosis is still poor despite of recent advances in treatment [3], it is urgent to decipher the molecular mechanisms underlying BE progression towards EA, in order to improve its diagnosis and treatment. In particular, it is important to search for new potential markers to better identify BE patients at risk for progression to EA.

For this purpose, several animal models have been developed, however none of them is devoid of some weakness [4]. Indeed, the ideal animal model should encompass all the steps between BE and EA, also modelling the chronic inflammation which underlies any step of esophageal carcinogenesis [5]. Moreover, to better model human BE, genetic heterogeneity should be taken into account for the validation of the animal model [6]. The first animal model for BE was proposed by Bremner in 1970 and it was developed in dogs [7]. In the 90s, several animal models were proposed; however, most of them are rat models while only a few mouse models have been proposed more recently. Mouse models are progressively substituting the rat, which is up to now the most used animal for BE models [8]. Indeed, a mouse model for EA, based on a surgical approach, has been recently described [9].

Mouse models usefulness in cancer research is due to several features, in particular mouse genome has been completely sequenced [10] and genetic manipulation is feasible. The aim of this paper was that of developing and evaluating a surgical mouse model for BE, which could further apply to genetic and pharmacological studies.

Materials and Methods

BE induction in mice

Experiments in CD1 mice were performed at the Laboratory of Genetic Engineering for the Production of Animal Models (L.I.Ge.M.A) at the Animal House of the University of Florence. 44 three-week-old CD1 mice were studied, represented by 39 operated according to the experimental model and 5 control (sham operated) mice at four different times. The characteristics of each series of mice are given in Table 1. Water and a standard chow were given until the day before surgery. Water was discontinued the morning of the operation. Mice were warmed by a hot lamp before and during the operation in order to prevent postoperative hypothermia. Anesthesia was performed by an intraperitoneal injection of Avertin (17 µl/g). All mice were operated on a small operating table and a 2.3-cm median laparotomy was
performed. After a wide divarication of both left liver lobe and left part of median lobe, esophagogastric junction was exposed. After sectioning of left gastric vessels, distal esophagus was sectioned after clamping in order to avoid esophageal retraction and the gastric side stitched with Goretex 8/0. Jejunum was prepared 5 cm after Treitz ligament and a small hole was performed in its wall by a 14 Fr intravenous cannula and an end to side esophago-jejunal anastomosis was performed either with Goretex 8/0 or silk 8/0 with the cut end of the esophagus. Abdominal wall was closed with nylon 4/0. As regard as the 5 control mice, a sham operation was performed. The same anesthesia protocol was applied as the other group and the operation consist in opening the abdomen and after an exploration excluding other concomitant pathologies, in closing it with nylon 4/0 as the other group. Liquid feeding was restored in the first post-operative day and solid feeding started the day after. All the animals which got sick or underwent a relevant weight loss were killed. In the control group mice a sham operation was performed. The experiment was ended after 10 months and mice were sacrificed. The study was conducted after the approval of the Ethical Committee, in accordance to the criteria outlined in the Guide for the Care and Use of Laboratory Animals.

Histological analysis

Samples were taken in the operating room of the animal facility immediately after the animal sacrifice. The entire stomach and esophagus were removed and were immediately fixed in formalin for at least 24 hours. Subsequently, samples were processed for paraffin embedding and 7 µm sections were obtained through a microtome and put on positive charged slides. Sections were then stained with Hematoxylin & Eosin following a standardised protocol and observed under a light microscope. The same samples were stained with Alcian Blue to detect goblet cells presence and an accurate examination of the sections was performed.

Results

Data reported in this paper were obtained performing an esophago-jejunal anastomosis in CD1 mice to establish a mouse model for BE.

Gastrojejunal anastomosis was performed with Goretex 8/0 in 20 mice and with silk 8/0 in 19 mice in order to identify the best surgical technique. No significant difference was found neither for manual ability requested nor for results in terms of Anastomotic leakage. A drawing showing the surgical procedure is reported in Figure 1A, while a representative picture showing the result of the surgical procedure is reported in Figure 1B.

Due to technical problems related to bleeding and/or anastomosis, the overall mortality rate was 11%. In particular, since in a first series animals suffered from hypothermia and anaesthesia problems, thereafter in addition to changes in the anaesthetic treatment, the temperature in the operation room was set at 30°C and animals were kept at the same temperature for 2 hours after surgery. In the third series survival rate was lower; this was probably due to the lower mean weight of the animals. In the last group a thermophore set at 37°C was used, in addition to the previous adjustments and survival rate was significantly higher (Table 1). The higher survival rate in the last group of animals was probably due to different reasons: 1) the temperature was kept at higher 7 values to avoid hypothermia; 2) low doses of 2.5% Avertin were used for anaesthesia; 3) animals were fed with semi-liquid food, obtained by homogenizing standard food with water, during the first three days after surgery.

Macroscopic evaluation, further corroborated by histological analysis of the samples, showed that animals belonging to the control group did not develop lesions, as witnessed by the presence of an intact Z-line to make the boundary between the squamous and gastric-type epithelia (Figure 2A). All the animals of the operated group showed the signs of gastric mucosal atrophy due to the esophago-jejunal

| Group | Number of Animals | Mean Weight (Range) | Mortality Rate (%) |
|-------|-------------------|---------------------|--------------------|
|       |                   |                     | Intraoperative | Perioperative | After 1 week |
| I     | 5                 | 28 g (26-30)        | 80              | 0            | 0           |
| II    | 15                | 29 g (27-31)        | 20              | 26.7         | 26.7        |
| III   | 12                | 24 g (21-26)        | 16.7            | 58.3         | 0           |
| IV    | 12                | 30 g (28-34)        | 8.3             | 16.7         | 8.3         |

Table 1: Characteristics and mortality rates of the different groups of animals.
anastomosis (Figure 2B). Moreover, 6 (14%) of the operated animals developed histologically detectable intestinal metaplasia in the lower esophagus. On the contrary, none of the controls animal developed atrophy or intestinal metaplasia in the lower esophagus.

When the presence of intestinal metaplasia was suspected, Alcian Blue staining was also performed, in order to detect goblet cells. The picture in Figure 3A shows the presence, at the gastroesophageal junction, of an area characterised by intestinal metaplasia. In Figure 3B a higher power microphotograph is reported, in which goblet cells can be better identified, stained in blue due to the presence of acid mucin which were selectively stained by Alcian Blue dye.

Discussion

In the past years, many progresses have been made in understanding the mechanisms of reflux esophageal injury which leads to BE and Barrett’s adenocarcinogenesis. However, human studies concerning the pathogenesis and relationship between the different steps of the carcinogenetic model obtain different results. It is well known that gastroesophageal reflux (GER) often causes peptic injury and inflammation of the esophageal squamous epithelium, a condition called reflux esophagitis. Esophagitis in most cases heals through the regeneration of squamous cells in the distal oesophagus. Conversely, in some patients esophagitis heals through a columnar-metaplastic process, which seems to represent a temptatively protective reaction to reflux injury since the metaplastic epithelium is more resistant to the noxious agents than the native tissue. In a minority of these cases intestinal-type columnar cells are represented; this condition is called Barrett’s esophagus (BE) and represents the most common esophageal pre-cancerous lesion. Therefore, the sequence of events-reflux-esophagitis-intestinal metaplasia (BE)–dysplasia-invasive cancer is widely accepted as the main carcinogenetic pathway in the esophagus. However the mechanisms that underlie this progression need to be defined. Despite many molecules have been widely studied such as COX-2 [11], Herg1 [12], bcl-2 and bcl-xl. [13], no conclusive studies are present literature. This is partly due to inter individual variability in humans and to the difficulties both in surveillance and in the interpretation of the endoscopic and histologic findings. For these reasons, the creation of a reproducible experimental model for BE (reviewed in [5]) is needed and can be useful to better understand the progression from BE to esophageal adenocarcinoma.

It is well known that, whereas humans might spontaneously develop BE, mice and rats do not, mainly because of differences in esophageal biology. For these reasons, surgery has been generally required for BE occurrence in rats and mice, although BE might be observed in animals treated with carcinogens, after esophagitis development [8]. Moreover, for the study of the molecular events involved in the carcinogenesis of Barrett’s carcino, a mouse model would be much more promising since most of the genetically altered animals are mice. However, no such mice models exist nowadays; it is probably due to the high mortality involved with the surgical procedure to create a mixed duodenogastric reflux and with the uncertain postoperative course of mice.

In a recent paper, a surgical approach similar to the one described in this paper was used [9]. However, several differences can be found between the two studies. First, mice included in the present study are 3 weeks old while in the cited paper are 6-8 weeks old. Moreover some differences could be found in surgical technique too, especially in the materials used for sutures, and in the food administration. Finally we have a control group which is necessary to demonstrate the effect of duodenal reflux in the development of BE.

Conclusion

The aim of the study was to demonstrate that the model we proposed could be applied in mice because of a technical feasibility.
and an acceptable mortality. In the literature many surgical procedure and the use of carcinogenic drugs have been reported. In spite of all the limitations of the present paper, our results show that this model can be used to study the development of reflux-induced esophagitis, BE and EA without using any drug. Therefore this model can be proposed also in engineered mice in order to better understand the role of duodenogastroesophageal reflux as well as the role of molecular markers in EA pathogenesis.

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