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Author/s:

Read, MD;Krishnadath, KK;Clemons, NJ;Phillips, WA

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Read *et al.*

Models of Barrett's carcinogenesis

Preclinical models for the study of Barrett's carcinogenesis

Matthew David Read,¹ Kausilia K. Krishnadath,² Nicholas J. Clemons,^{3,4} and Wayne A. Phillips^{3,4}

¹Cancer Biology and Surgical Oncology Laboratory, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia. ²Department of Gastroenterology and Hepatology, Academic Medical Center, Amsterdam, the Netherlands. ³Division of Cancer Research, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia. ⁴Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, Victoria, Australia

Address for correspondence: Matthew David Read, Cancer Biology and Surgical Oncology Laboratory, Peter MacCallum Cancer Centre, 305 Grattan Street, Melbourne, VIC 3000, Australia. mattread80@gmail.com

Barrett's esophagus (BE) is clinically significant, as it is the only known precursor lesion for esophageal adenocarcinoma. To develop improved therapies for the treatment of BE, a greater understanding of the disease process at the molecular genetic level is needed. However, achieving a greater understanding will require improved preclinical models so that the disease process can be more closely studied and novel therapies can be tested. Our review highlights progress in the development of preclinical models for the study of BE and identifies the most suitable model in which to test novel therapies.

Keywords: Barrett's esophagus; reflux models; transgenic models; organoid; preclinical model; animal models

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Introduction

Barrett's esophagus (BE) is the eponym describing the metaplastic process that occurs in the lower esophagus in response to gastroesophageal reflux (GER). BE is clinically significant because it is the only known precursor lesion for esophageal adenocarcinoma (EAC), a cancer with an extremely poor prognosis and an incidence that has risen more than sixfold over the last four decades.¹ Unfortunately, the exact mechanisms responsible for both the initiation of BE and its progression to EAC are poorly understood, and this has led to several key controversies in the field. One of the main controversies surrounds the location and identity of the Barrett's cell of origin.^{2,3} Current theories include cells that are native to the esophagus (squamous cells, ductal cells, and submucosal gland cells), cells located at the squamocolumnar junction (SCJ) (transitional basal cells and embryonic stem cells), cells from the proximal stomach (gastric cardia), and even bone marrow-derived cells.⁴ Another controversy, closely entwined with the Barrett's cell of origin, is whether BE forms through a process of transdifferentiation (transformation of one differentiated cell type to another) or transcommitment (reprogramming at the stem or progenitor cell level).³ Several controversies regarding the pathogenesis of Barrett's carcinogenesis also exist at the molecular genetic level. Examples include the component of refluxate responsible for the initiation of the metaplastic process,⁵ as well as the sequence of mutations and epigenetic changes required for neoplastic progression.^{6,7}

Current treatments for BE consist of endoscopically resecting or ablating metaplastic segments using a combination of either endoscopic submucosal dissection, endoscopic mucosal resection, or radiofrequency ablation.⁸ These treatments aim to denude portions of the esophagus, which in the absence of the GER, undergo repair with neosquamous epithelium.⁸ Unfortunately, these techniques can lead to both recurrent Barrett's⁹ and buried Barrett's,¹⁰ making ongoing surveillance and treatment challenging. These techniques can also lead to complications such as perforation, stricture formation, and bleeding. In a recent systematic review, assessing the safety of endoscopic treatments for both BE and early EAC, the rates of perforation, stricture formation, and bleeding were 33.5%, 7.5%, and 1.3%, respectively.¹¹ There is currently no pharmacotherapy that targets the molecular drivers of BE, either for the prevention or treatment of the disease.

To develop improved therapies to treat BE, a greater understanding of the disease processes at the molecular genetic level is required. However, for therapeutic development to occur, improved preclinical models are required for mechanistic studies and testing of novel therapies.¹² The ideal model for the study of Barrett's carcinogenesis should be manipulable, reproducible, and three-dimensional and should allow for environmental exposures. As BE is likely due to a complex

interaction between different cell types,¹³ the ideal model should include a full complement of epithelial, stromal, and inflammatory cells.¹⁴ The epithelial component should include cells from the esophagus (including submucosal glands and ductal cells), stomach, and gastroesophageal junction. Through such an approach, all potential sources of the BE cell of origin should be included. The ideal model also needs to undergo strict validation to confirm that it recapitulates the human disease process and can model the progression from the BE cell of origin to columnar epithelia to adenocarcinoma (AC). Our review highlights the progress made in the development of preclinical models for the study of BE.

Methodology

A PubMed search was performed for publication dates from January 1950 to March 2018 using a combination of the following terms: Barrett's esophagus, preclinical model, reflux model, animal model, transgenic model, cell line, organotypic model, and organoid model. Articles were limited to those published in English. A manual search of citations for relevant articles was also performed. Both original articles and review articles were included.

***In vivo* models**

While a number of key discoveries have been published from a range of different preclinical models of BE, there have also been a number of conflicting results. These conflicting results were observed because there is no single preclinical model that offers the ideal system with which to study Barrett's carcinogenesis.¹⁵ While attempts have been made to develop an *in vivo* Barrett's model from several different species, research efforts have mainly focused on murine and canine models. To generate both the Barrett's phenotype and EAC within these animal models, a number of modifications have been attempted. These include the surgical induction of different types of reflux, genetic modification, and exposure to different forms of exogenous carcinogens.^{16–19}

Research in this field was originally focused on reflux esophagitis and the columnar lined esophagus (CLE). During this early period, several studies were performed using canine models to investigate the effects of various refluxates on the esophagus. An advantage of this model is that both the canine and human esophagi contain submucosal glands.¹⁵ In a landmark study by Bremner *et al.*, dogs were randomized to one of three groups following stripping of the lower esophageal mucosa. These included a surgically induced reflux group, a gastric hypersecretion group, and a control group.²⁰ The results indicated that neo-epithelialization occurred via a columnar epithelium in the reflux group, whereas a squamous epithelium predominated in those animals with a competent

lower esophageal sphincter. From these results, the authors concluded that the CLE is an acquired condition formed from the migration of columnar cells from either a gastric or a junctional origin. In 1988, Gillen *et al.* published a similar study to that performed by Bremner *et al.*²¹ However, in that study using a canine model, the lower esophageal mucosa was denuded in a certain manner to create a squamous barrier to any migrating gastric or junctional cells. Similar to Bremner, the authors observed the formation of a columnar epithelium in the presence of acid reflux, both on its own and in combination with bile (but not bile on its own). From these results, they also concluded that BE is an acquired condition. However, unlike Bremner, the authors concluded that the columnar cells originate from a source intrinsic to the esophagus, such as the submucosal gland. These results were further supported by canine studies conducted by Li *et al.*, who concluded that the regenerating epithelium was in continuity with the ducts of the esophageal glands.²²

Two of the seminal studies assessing the effect of refluxate composition on the esophagus were Ferguson *et al.* in 1950²³ and Redo *et al.* in 1959.²⁴ Ferguson *et al.* demonstrated that either the combination of acid and pepsin or bile on its own is capable of causing esophagitis. However, when the lower esophagus is perfused with acid on its own, no effect is seen. In the study by Redo *et al.*, canine esophagi were perfused with different types of refluxate over a seven-and-a-half-h period. The refluxates included gastric juice, hydrochloric acid, pepsin, and bile, which were collected from a patient with a duodenal fistula. The results were consistent with those obtained by Ferguson *et al.*, suggesting that acid-pepsin activity is responsible for ulceration of the esophagus. However, in this study, no effect was seen with bile reflux. These results contradicted earlier observations made by Barrett regarding the destructive effects of bile. In his 1954 paper, Barrett described the severe esophagitis experienced by one patient following a total gastrectomy and esophagoduodenostomy.²⁵

To further investigate the effects of reflux composition on the development of esophagitis, Levrat *et al.* devised a surgically induced reflux model in the rat.¹⁶ This study followed on from previous work investigating reflux esophagitis in the rat following total gastrectomy.^{26,27} In Levrat *et al.*'s study, six different types of anastomosis were fashioned to induce different types of reflux. These consisted of an esophagojejunostomy with different combinations of gastrectomy and pancreaticobiliary diversion. The different approaches enabled the study of gastric, biliary, and pancreatic secretions both in isolation and combination. The results suggested that bile was the key injurious factor and that gastric secretions alone had little effect. Despite key anatomical and histological differences between the murine and human esophagus, such as the presence of a keratinized squamous epithelium within the esophagus, an SCJ located within the forestomach, and the absence of

esophageal submucosal glands (ESMGs),¹⁵ this paper served to popularize the murine model and formed the basis from which several later surgical reflux models were generated.^{28–35}

Once the association between the CLE and EAC became apparent, research shifted from investigating reflux esophagitis to the more clinically important metaplasia–dysplasia–carcinoma sequence. Given the complexities involved in such a disease process and its long natural time course, researchers have gone to great efforts to generate a suitable animal model. Potential advantages of animal models include the ability to adjust for the known risk factors, such as sex and obesity.¹⁵ These models also have the ability to mount an inflammatory response, which is likely to be one of the key early steps in the pathogenesis of BE.³⁶ Such a model could allow investigation into each of the various stages of the malignant transformation and provide the opportunity to test both preventative and treatment strategies in a controlled fashion.³⁷ Unfortunately, despite many attempts over the last two to three decades, the ideal animal model to Barrett’s carcinogenesis has not been identified. This is largely due to animals rarely developing a natural BE phenotype.¹⁵ In fact, there have only been three reported cases of animals developing spontaneous BE, two of which were in dogs^{38,39} and one in a baboon.⁴⁰ Of the two cases reported in dogs, one also progressed to EAC.³⁸ Despite both the dog and baboon developing BE spontaneously, they failed to become popular as preclinical models because they are resource intensive and can take up to 30 months to develop BE⁴¹ and 60 months to develop EAC.¹⁵

Despite its limitations, the bulk of the research has been conducted using the surgically induced reflux murine model to determine if both BE and EAC will develop under augmented conditions. In early studies, the added effect of carcinogen administration was tested in combination with the surgical induction of reflux.^{19,42–44} The results from these studies revealed that reflux on its own could induce esophagitis, metaplasia, and cancer formation. However, the rates increased with carcinogen exposure. Clark *et al.*¹⁹ reported that esophagitis occurred in 97%, metaplasia in 10%, dysplasia in 8%, and cancer in 3% of the cases. However, unlike humans, the cancers were squamous cell carcinomas and not ACs. When combined with carcinogen exposure, these rates increased to 99% for esophagitis, 13% for metaplasia, and 57% for carcinoma. In this setting, both squamous cell carcinomas and ACs developed at a ratio of approximately two to one. The requirement for carcinogen exposure to produce ACs of the distal esophagus was challenged in later studies.^{45–47} Fein *et al.* reported AC formation without the need for carcinogen.⁴⁷ In this series, all animals exposed to duodenal reflux demonstrated evidence of severe esophagitis, 87% had evidence of a columnar lined distal esophagus, and 48% had AC of the anastomotic site. Goldstein *et al.* also reported AC formation in the absence of carcinogen. In their study, a higher rate of AC formation (73%) was

achieved simply by correcting the iron deficiency anemia that occurs following the surgical induction of reflux.⁴⁶ This rate was not affected by the administration of carcinogen, which promoted the formation of squamous cell carcinomas instead.

To extrapolate the results from animal models to the human disease process, it is essential that models undergo rigorous validation.³⁰ Unfortunately, the results from multiple studies aiming to validate the surgically induced reflux model have varied, leaving two fundamental issues unresolved. The first issue is whether the CLE seen within the model is truly metaplastic or simply represents the migration of epithelial cells from below the anastomosis. The research supporting the metaplasia hypothesis has provided evidence that the CLE within the model shares similar morphology, mucin profile, and markers of differentiation (CK7, CK20, Das-1, villin, and pS2/TFF1) to BE.⁴⁸ Further studies have also been performed comparing the trefoil factor (TFF) gene expression profile between the CLE and jejunum distal to the anastomosis, as TFF has previously been validated as a marker capable of discriminating between BE and small intestine.⁴⁹ The results revealed a statistically significant higher expression of TFF-1 and TFF-2 within the CLE compared with that in the jejunum.³⁴ Gronnier *et al.* later confirmed these results.³⁰ However, they also identified Brunner's glands within the columnar lined esophageal segment. Because these glands normally reside within the submucosa of the first part of the duodenum, this raised the possibility that duodenal tissue may have been mechanically introduced into the distal esophagus, suggesting that both mechanisms may be occurring.³⁰ Buskens *et al.* also suggested that the ectopic glandular tissue could be implanted into the esophageal submucosa following an anastomosis, leading to AC formation.³⁷

The second unresolved issue is whether the ACs that develop within the model are representative of those seen in humans. With respect to the histology and invasive potential of these tumors, most studies have reported the development of well-differentiated mucinous tumors at the level of the anastomosis.^{35,37,50} Microscopically, these tumors appear to originate from the submucosa and extend to the external surface, lacking any mucosal involvement. They also exhibit a benign phenotype with no evidence of either local or distant dissemination.^{37,50} This phenotype is in vast contrast to most human ACs, which display varying degrees of differentiation, arise from the mucosa, and frequently metastasize.⁴⁸ In contrast to these findings, Su *et al.* reported that 12 out of 14 cases of the EAC in their series demonstrated evidence of invasion into muscle, adventitia, and adjacent organs, although these findings were not readily apparent in the published images.⁴⁸ Gronnier *et al.* also described the presence of distant lung and liver metastases from their series in previously unreported data detailed in a letter to the editor.⁵¹ In an attempt to validate these tumors at the molecular level, Su *et al.* demonstrated that the immunohistochemical expression of tumor

markers p53 and COX-2 within the reflux-induced ACs was comparable to that of human controls.⁴⁸ In contrast, Buskens *et al.* reported that p53 immunohistochemistry was not associated with malignant transformation within the model.³⁷

Transgenic models

Although the exact mechanisms responsible for the development of BE and its subsequent progression to EAC remain unknown, it is likely that the disease involves a complex interplay between molecular and genetic events, cellular interactions, and environmental exposures. Given these complexities, numerous genes and signaling pathways have been implicated in its pathogenesis. Examples include genes involved in intestinal differentiation, such as the homeobox genes CDX1 and CDX2, and signaling pathways involved in both esophageal embryogenesis and intestinal development, including bone morphogenetic protein 4, sonic hedgehog (Shh), Notch, and Wnt.^{18,52,53} With the advent of the genetically engineered mouse models, a unique opportunity was presented to test the functional effect of individual genetic alterations in a controlled setting.

Unfortunately, despite initial optimism, genetically engineered mouse models still have significant limitations. First, these models still possess all the same limitations of mouse models that have previously been discussed. Second, the anticipated phenotype is not always achieved.⁵⁴ This is especially relevant for complex disease processes, such as Barrett's carcinogenesis, where multiple factors are involved in disease pathogenesis. In this instance, the addition of reflux may be required to generate the desired phenotype, as demonstrated by Fein *et al.*⁵⁵ Both factors make it difficult to interpret the results and relate them to the human disease process.

Despite these shortcomings, several key studies have attempted to investigate the fundamental questions related to the pathogenesis of BE using transgenic models. Such areas include the cell of origin of BE and the role of inflammation and signaling pathways in the pathogenesis of BE.

In a study by Quante *et al.*, a transgenic mouse model was used to assess the effect of esophageal inflammation on the Barrett's carcinogenesis sequence.¹⁷ In this model, interleukin (IL)-1 β , a proinflammatory cytokine upstream of IL-6 and TNF- α , was overexpressed. This led to a Barrett's-like metaplasia at the SCJ, representative of the human disease. Through lineage tracing experiments, the authors also concluded that the metaplasia was due to the migration of Lgr5+ cells from the gastric cardia.¹⁷

Another signaling pathway implicated in the pathogenesis of BE is the Hedgehog pathway, which drives the development of the CLE during embryogenesis.³⁶ To test if the reactivation of this

pathway is sufficient to induce a columnar phenotype in the adult esophagus, Wang *et al.* used a conditional Shh transgenic model.¹⁸ Following transduction, esophageal epithelial cells were cocultured with fibroblasts within an *ex-vivo* transplantable culture system. While Shh expression failed to generate an overt columnar epithelium in this model, it did lead to the expression of the columnar markers cytokeratin 8/18 and Sox9.

In a study addressing the cell of origin of BE, Wang *et al.* elected to use the p63-deficient mouse in an attempt to model the damage caused by reflux.⁵⁶ As p63 is required for the self-renewal of stem cells within stratified epithelium, these mice fail to generate stratified epithelial tissues and can only survive *in utero*. By assessing the embryonic tissue, the authors claimed that these mice generated Barrett's-like metaplasia, consistent with the human disease and that the columnar cells originate from residual embryonic cells located at the SCJ.⁵⁶ However, a criticism of this study is that the authors were simply studying a defect of embryogenesis rather than true metaplasia and that their conclusions may not be valid. More recently, however, Jiang *et al.* have challenged these findings.⁵⁷ Jiang *et al.* were the first group to identify a transitional zone at the SCJ in both humans and mice. This transitional zone is characterized by basal cells that express both squamous (p63, KRT5) and columnar (KRT7) markers and suprabasal cells that only express columnar markers. Lineage tracing confirmed that these KRT7 positive suprabasal cells originated from the basal layer of the transitional epithelium. Using a mouse model that was genetically engineered to express the intestinal transcription factor CDX2, this transitional zone was shown to expand. This led to a gradual decrease in squamous markers and an increase in columnar markers, typical of the human disease process.

Genetically engineered mouse models have also been combined with surgically induced reflux models in an attempt to investigate the role of both candidate genes and environmental exposures within a controlled system. However, it was precedent on the successful generation of the reflux model within the mouse. The first report of such a model was in 1999 by Fein *et al.*⁵⁵ In this study, a total gastrectomy was performed in conjunction with an esophagojejunostomy on the wild type, p53 knockout or APC-mutated mice. The results revealed that both p53 loss and APC-mutation were associated with the formation of a CLE, whereas only p53 loss was associated with progression to AC. Xu *et al.* later conducted further work assessing the feasibility of the surgically induced reflux model in mice.⁵⁸ In that study, the authors reported an extremely low operative mortality rate of only 7.5%. Consistent with earlier studies conducted in rats, both CLEs and carcinomas were evident. The carcinomas were predominantly either squamous or adenosquamous, and their rate of formation increased significantly with additional carcinogen administration. Raggi *et al.* also

conducted similar work assessing the suitability of the mouse model.³³ However, they reported a much higher incidence of AC formation without the need for carcinogen. Given the steep learning curve and difficulties associated with operating on small animals,³³ an alternative technique has been developed that employs micromagnets to induce fistula formation.⁵⁹

***In vitro* models**

In vitro models are tools in which cells are cultured and studied outside of their native environment. To study BE, cells are typically derived from either the normal esophagus or regions of BE. However, as cells derived from freshly digested tissue tend to have a finite lifespan, the majority of research has focused on cells that have been immortalized. The successful culture of cells also requires the establishment of an artificial environment that attempts to recreate the *in vivo* conditions. Examples include the simple two-dimensional culture of cells on plastic to more complex coculture systems that recreate a three-dimensional environment. More recently, organoid models have gained popularity in the field of Barrett's carcinogenesis.

Cell lines

In addition to established cancer cell lines, a number of immortalized epithelial cell lines have been generated from both normal esophageal tissue and BE, creating the potential to study multiple stages of the metaplasia-carcinoma sequence in the *in vitro* setting. The first such cell line, HET-1A, was developed in 1991.⁶⁰ In this study, normal esophageal tissue was collected at autopsy and cultured under serum-free conditions using an explant technique. Epithelial cells were then immortalized following transfection with viral genes (simian virus 40 large T-antigen).⁶⁰ An alternative technique for cell immortalization is via the expression of hTERT, the catalytic subunit of telomerase.⁶¹ hTERT acts to maintain the lengths of the telomeres, which are protective structures at the end of chromosomes. Under normal conditions, telomeres undergo shortening with each cell division and, once a critical length is reached, trigger a permanent cell cycle arrest.⁶¹ Using this technique, numerous normal esophageal and Barrett's cell lines have been created.^{15,61} As an extension of the simple *in vitro* culture of established cell lines, numerous studies have augmented the culture conditions through the addition of both acid and bile salts.^{62,63} While there are no standardized culture conditions, the addition of acid and bile salts has been demonstrated to induce BE-specific molecules.⁶²

Unfortunately, established cell lines are clonal, and the identity of their progenitor cell is unknown, limiting their use in the study of Barrett's carcinogenesis.¹⁵ In theory, for a cell line to be of value in the study of Barrett's carcinogenesis, it needs to be derived from the Barrett's cell of origin, which

could include any number of sources, such as squamous cells, ductal cells, cells from the ESMG, transitional cells, or gastric cardia cells.⁴

As the stroma has been reported to play a crucial role in the pathogenesis of BE,¹⁸ *in vitro* assays based purely on epithelial cell lines may also be ignoring key interactions with the microenvironment. In an attempt to overcome this limitation, the more complex organotypic model was developed.

Organotypic models

The “organotypic assay” was originally published in 1983 and was developed to better recapitulate the *in vivo* conditions of epithelial cells. Through the use of a collagen basal layer and an air–liquid interface, it was demonstrated that cultured skin keratinocytes could form a three-dimensional polarized epithelium both *in vitro* and *in vivo* using a transplantable culture system.⁶⁴ However, it took another 20 years before a group led by Rustgi adapted this model for the study of esophageal epithelium.⁶⁵ In this study, the model was modified to include the seeding of fibroblasts within the collagen matrix before it was subsequently used to assess the effect of epidermal growth factor receptor overexpression on epithelial homeostasis. Over the course of the following decade, Rustgi authored numerous other studies based on this model, including both methodological and mechanistic studies.^{12,66–70} Using this model, researchers have attempted to investigate the factors related to the pathogenesis of the columnar metaplasia seen in BE. These factors include signaling pathways and transcription factors such as Wnt and Cox2,⁷¹ Cdx1 and c-Myc,⁶⁹ in addition to the role of acid exposure.¹² Although these studies reported an upregulation of intestinal markers and varying degrees of mucin production, an overt Barrett’s phenotype, consisting of a single layer of columnar cells, was not achieved. The organotypic model has also been used as a tool to promote the *in vitro* differentiation of intestinal stem cells.⁷² This characteristic is thought to be the result of enhanced oxygen delivery to the cells and occurs because the thin film of media used within the model provides only a minimal barrier for gas diffusion.⁷³

In addition to the culture of normal keratinocytes, the organotypic model can also be used to culture cancer cells. To investigate the role of the stroma on the invasiveness of OAC, Underwood *et al.* cultured the established cancer cell lines Flo-1 and OE33 in combination with either normal or cancer-associated fibroblasts.⁷⁴ The results from this study revealed an increase in tumor cell invasion in the presence of cancer-associated fibroblasts, partly due to the matricellular protein periostin.

Organoid models

Organoids are complex structures that resemble their tissue of origin and are grown *in vitro* from individual stem cells.⁷⁵ Sato *et al.* first described the model in their seminal paper entitled “Long-term expansion of epithelial organoids from human colon, adenoma, AC, and Barrett's epithelium.”⁷⁶ The key to the development of this model was the identification of the essential factors required for stem cell maintenance. These factors include R-spondin (Wnt agonist), Noggin (BMP inhibitor), epidermal growth factor, and Matrigel to recreate the laminin-rich crypt microenvironment.⁷⁶

The great power of this model is that it can reconstitute a complete Barrett's phenotype from individual stem cells obtained from fresh biopsies, negating the use of established cell lines for preclinical studies. As organoids provide an ongoing tissue resource, there is also great potential to establish organoid biobanks for sharing of resources among researchers and replication of results. Compared with cell lines, organoids also provide a much better model of the molecular and genetic diversity seen within the human disease. Unfortunately, while squamous organoids derived from the murine esophagus have been readily cultured using this model,^{76,77} researchers have failed to develop a reproducible technique for the culture of squamous organoids derived from the human esophagus. However, encouragingly, researchers have recently optimized the generation of squamous organoids from the porcine ESMG.⁷⁸ Given the histological similarities with the human esophagus, efforts should now be made to generate squamous organoids from the human esophagus using similar techniques.

Summary

This review highlights the range of preclinical models that have been used for the study of Barrett's carcinogenesis (summarized in Table 1). It also identifies a number of key discoveries that have been made using these model systems. While the recently developed organoid models show great promise, it is clear that the ideal model for the study of Barrett's carcinogenesis is lacking. Thus, the choice of model system needs to be tailored to the hypothesis being investigated. Researchers may even find it prudent to use multiple models when answering a research question. For example, the organoid model may be used as a screening tool to assess potential compounds before progressing to animal models and, if successful, further progressing into clinical trials. Hopefully, such an approach will lead to improved patient outcomes.

Competing interests

The authors declare no competing interests.

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Table 1. Preclinical models of Barrett's carcinogenesis

Model	Advantages	Disadvantages
<i>In vivo</i>		
Murine	– Cost – Manipulable – Reflux model – Transgenic model	– Fails to demonstrate a natural BE phenotype without significant manipulation – Key anatomic and histologic differences with respect to the human (absence of ESMG, presence of a keratinized squamous epithelium, and SCJ located in the forestomach)

	– Environmental exposure – Ability to test the functional effect of different genes and exposures in a controlled setting	– Expected phenotype is not always achieved – Tumors tend to demonstrate a phenotype different from that of human tumors (benign appearance, submucosal, increased rate of ESCC, mucinous, and fail to metastasize) – Technically difficult to generate surgically induced reflux models
Canine	– Presence of ESMG – Reported to develop a natural BE and EAC phenotype – Can be combined with a surgically induced reflux model	– Large animal and requires significant resources – Length of time to develop BE and EAC (up to 5 years) – Unable to be genetically manipulated
<i>In vitro</i>		
Cell lines	– Cost – Reproducibility	– Lack other essential cellular elements (e.g., stromal and inflammatory cells) – Two-dimensional – Lack heterogeneity – Typically require established or immortalized cell lines
Organotypic	– Three-dimensional – Can assess the effect of cell interactions – Manipulable	– Lacks other essential cellular elements (e.g., stromal and inflammatory cells) – Unable to generate a true BE phenotype
Organoid	– Reproducible – Three-dimensional – Ongoing tissue resource	– Lacks other essential cellular elements (e.g., stromal and inflammatory cells) – Unable to culture human squamous organoids

ESMG, esophageal submucosal gland; SCJ, squamocolumnar junction; BE, Barrett's esophagus; EAC, esophageal adenocarcinoma; ESCC, esophageal squamous cell carcinoma.