Experimental models for Barrett’s esophagus and esophageal adenocarcinoma

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Garman KS, Orlando RC, Chen X. Experimental models for Barrett’s esophagus and esophageal adenocarcinoma. Am J Physiol Gastrointest Liver Physiol 302: G1231–G1243, 2012. First published March 15, 2012; doi:10.1152/ajpgi.00509.2011.—Several different cell culture systems and laboratory animal models have been used over the years to study Barrett’s esophagus (BE) and esophageal adenocarcinoma (EAC). Most of the existing models have key differences with the human esophagus and complex pathogenesis of disease. None of the models offers an ideal system for the complex study of environmental exposure, genetic risk, and prevention strategies. In fact, different model systems may be required to answer different specific research questions about the pathogenesis of BE and EAC. Given the high mortality associated with EAC and the fact that current screening strategies miss most cases of EAC, advances in basic and translational science related to esophageal injury, repair, and carcinogenesis are clearly needed. This review describes several of the existing and potential model systems for BE and EAC with their benefits and disadvantages.

Animal disease models; culture techniques; mice; rat; guinea pig; esophageal neoplasms

ESOPHAGEAL CANCER continues to be one of the deadliest cancers that humans develop, and despite screening, surveillance, and ablation programs for Barrett’s esophagus (BE), the incidence of esophageal adenocarcinoma (EAC) continues to increase (13). The relationship between underlying complex genetics and environmental exposures that lead to EAC remains incompletely understood. Observational studies in humans have improved our understanding of associations of BE, esophageal cancer, and clinical risk factors, yet the causality and mechanisms behind these interactions remain unclear.

An experimental model system of BE and EAC would allow detailed molecular and cellular assessment of clinical and genetic risk factors in the development of BE and EAC. Without such an experimental system, it will be difficult to discern a detailed understanding of the interplay between risk factors and underlying mechanisms of disease. Similarly, the lack of a solid experimental model for BE and EAC makes it more difficult to study preventive and treatment strategies.

Several different systems and organisms have been tested as models for BE and EAC, yet no one model offers an ideal system for the study of environmental exposure, genetic risk, and prevention strategies. Generally, in this review, BE will be considered to be the presence of columnar epithelial cells with goblet cells in the esophagus. Some of the animal models that have been developed result in creation of columnar epithelium without the goblet cells and may represent a partial model of BE.

In our review of experimental models of BE, it is important to acknowledge the controversy that surrounds the cellular origin of BE, and, in fact, there may be more than one source of cells in the esophageal region capable of transformation into columnar epithelium (9, 35). Some authors promote the idea of transdifferentiation of esophageal squamous cells into a columnar phenotype, focusing on the basal cells of the squamous esophagus, including CD34-positive cells in mice (62, 115). Other authors have promoted the concept that cells capable of generating columnar epithelium come from subepithelial stromal cells (18), such as esophageal ducts (78). It is also possible that cells originating from the bone marrow may contribute to esophageal metaplasia, as found in bone marrow transplant models (108). The controversy related to cell origin in BE is relevant for research involving existing or new models of BE and EAC, because the esophageal cells included in the models should represent cells with the potential to contribute to BE. In this review, we describe several of the existing and potential model systems for BE and EAC with their benefits and disadvantages.

Cell Culture-Based Methods

Cell culture has been used in several forms for the study of Barrett’s metaplasia. Simple in vitro models allow cells to be studied on an individual level, yet they lack the complexity of a multicell system.

Simple in vitro models. Squamous and Barrett’s esophageal epithelial cells can be grown in culture (135). Squamous epithelium can be represented by squamous cells grown in culture. Yet Barrett’s epithelium comprises a multiplicity of different types of cells, and culture systems of Barrett’s epithelium generally select a single cell type, limiting the in vitro model’s ability to reflect the diversity of cell types observed in Barrett’s columnar epithelium in vivo.

Typically, human squamous cells from the esophagus do not live in culture for >1 wk (109). However, primary rabbit esophageal epithelial cells have been maintained in culture for ~3 wk (122). When obtained from endoscopic biopsies, BE epithelial cells placed in Matrigel (BD Biosciences, Becton Dickinson, Franklin Lakes, NJ) and grown in significantly augmented medium (containing murine EGF, murine Noggin, human R-spondin-1, recombinant human Wnt-3A, gastrin, nic-
otinamide, A83-01, and SB-202190) have been reported to live for ∼1 mo (109). With the addition of fibroblast growth factor, cells demonstrate budding and can survive for >3 mo (109). In these culture conditions, organoids from Barrett’s epithelium are Ki67-positive and demonstrate multilineage differentiation, with some mucin-positive cells and some lysozyme-positive Paneth cells (109).

Immortalized cell lines derived from human esophageal epithelium are also commercially available. Immortalized cell lines offer the benefit of reproducible growth of different types of esophageal epithelial cells. Squamous and Barrett’s cells have been immortalized. HET-1A cells (American Type Culture Collection, Manassas, VA) are an SV-40 immortalized cell line of human squamous esophageal epithelial cells (95). It has been argued that SV-40 immortalized cells may demonstrate changes in cell cycle checkpoints and increased genomic instability, making them less preferable to telomerase-immortalized cells. HET-1A cells have been found in organotypic culture to lack normal squamous morphology and to appear dysplastic and proliferative, with hyperchromic nuclei, vacuole formation, and increased Ki67 staining (123). The HET-1A cells lacked evidence of normal squamous differentiation, such as E-cadherin and keratin 5/6 expression, while expressing mesenchymal markers, such as vimentin and N-cadherin (123). Similar findings were reported by Green et al. (41): in organotypic culture, HET-1A cells did not produce stratified squamous epithelium with maturation, as is typical of squamous epithelium, and markers keratin 4 and involucrin were absent.

As an alternative, telomerase-immortalized esophageal squamous cell lines generally are more representative of typical squamous morphology, and they have been described by several groups (24, 49, 53, 63, 85, 143). Telomerase-immortalized BE cells have also been described (51, 55, 96). Nonmalignant immortalized esophageal cell lines are listed in Table 1.

Huo et al. (53) found differences in gene expression patterns of squamous esophageal cells derived from patients with and without BE. Recently, Wang et al. (130) presented data that squamous esophageal cells from patients with BE were more likely to express cytokeratins typically found in Barrett’s epithelium: cytokeratin 8 and cytokeratin 18. Knocking down SRY (sex-determining region Y)-box 9 (SOX9) in this model led to decreased expression of cytokeratins associated with BE (130).

One of the challenges with squamous esophageal cell lines is that the cell layer of origin of these cell lines (basal vs. more superficial) is not known. The origin of the cells is important, because the location of the progenitor cell in the esophagus that provides the source of the BE cells remains unclear. Indeed, it remains a possibility that Barrett’s epithelium is derived from the ducts of esophageal submucosal glands.

One advantage of the in vitro models is the potential to investigate genes that demonstrate certain patterns of expression in vivo in human esophageal samples and then, with relative ease, to target those specific genes in a controlled culture setting to evaluate genetic function in the context of pathways. Peng et al. (98) described in vitro models that were used to evaluate function of glutathione peroxidase 7, one of the genes that may be silenced through epigenetic mechanisms in BE and EAC. In BAR-T (immortalized BE cell lines), when adenovirus was used to express glutathione peroxidase 7, cells retained viability after exposure to hydrogen peroxide compared with control (98).

It is likely that BE develops based on coordinated signaling between overlying epithelium and underlying stroma, which includes fibroblasts, myofibroblasts, endothelium, neurons, and inflammatory cells beneath the epithelium (92). Because of the inherent two-dimensional nature, a disadvantage of standard in vitro techniques is the inability to allow for normal cellular organization, stratification, polarization, junction development, or interactions with underlying stroma.

These interactions between epithelium and stroma may also be important for the development of EAC. Several studies describe the relationship between stroma and epithelium in BE and EAC, although the complexity of the cross talk remains incompletely understood (77, 107, 129). Simple models of cell culture lack this complex interplay between the esophageal layers.

Organotypic culture. To address the lack of complexity in simple cell culture systems, more complex coculture systems that mimic the in vivo interplay between epithelium and underlying stroma have been developed. Rustgi and colleagues (5, 62) developed the organotypic culture system that allows growth of epithelial cells and stromal cells in concert. It is thus possible to study proliferation and histology of the squamous epithelium, as well as interplay with supporting cells. The organotypic culture system has been used for the study of different fibroblast types and the role of fibroblasts in tumor development (94). However, the formation of Barrett’s epithelium from human esophageal keratinocytes has proved elusive.

Table 1. Nonmalignant immortalized esophageal epithelial cell lines, cell types, and immortalization techniques

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Cell Type</th>
<th>Immortalization Technique</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HET-1A</td>
<td>Squamous epithelium</td>
<td>SV-40 immortalized</td>
<td>24</td>
</tr>
<tr>
<td>NE2-hTERT</td>
<td>Squamous epithelium</td>
<td>hTERT immortalized</td>
<td>49</td>
</tr>
<tr>
<td>G2T</td>
<td>Squamous epithelium (no BE in patient)</td>
<td>hTERT immortalized</td>
<td>53, 143</td>
</tr>
<tr>
<td>G4T</td>
<td>Squamous epithelium (no BE in patient)</td>
<td>hTERT immortalized</td>
<td>53, 143</td>
</tr>
<tr>
<td>B3T</td>
<td>Squamous epithelium from BE patient</td>
<td>hTERT immortalized</td>
<td>53, 143</td>
</tr>
<tr>
<td>B10T</td>
<td>Squamous epithelium from BE patient</td>
<td>hTERT immortalized</td>
<td>53, 143</td>
</tr>
<tr>
<td>EPC2-hTERT</td>
<td>Squamous epithelium</td>
<td>hTERT immortalized</td>
<td>96</td>
</tr>
<tr>
<td>CP-A hTERT</td>
<td>Columnar epithelium from BE patient</td>
<td>hTERT immortalized</td>
<td>96</td>
</tr>
<tr>
<td>CP-B hTERT</td>
<td>Columnar epithelium from BE patient</td>
<td>hTERT immortalized</td>
<td>96</td>
</tr>
<tr>
<td>CP-C hTERT</td>
<td>Columnar epithelium from BE patient</td>
<td>hTERT immortalized</td>
<td>96</td>
</tr>
<tr>
<td>CP-D hTERT</td>
<td>Columnar epithelium from BE patient</td>
<td>hTERT immortalized</td>
<td>96</td>
</tr>
<tr>
<td>BAR-T</td>
<td>Columnar epithelium from BE patient</td>
<td>hTERT immortalized</td>
<td>51, 55, 143</td>
</tr>
<tr>
<td>BAR-T 9</td>
<td>Columnar epithelium from BE patient</td>
<td>hTERT immortalized</td>
<td>51, 55, 143</td>
</tr>
<tr>
<td>BAR-T 10</td>
<td>Columnar epithelium from BE patient</td>
<td>hTERT immortalized</td>
<td>51, 55, 143</td>
</tr>
</tbody>
</table>

BE, Barrett’s esophagus; hTERT, human telomerase reverse transcriptase.
using organotypic model (115). The organotypic system has been used to evaluate the self-renewal capacity of potential esophageal stem cell populations (62). Given the structure associated with Matrigel, transition to the cancer phenotype, including invasion capability, can be assessed after manipulation of cell populations (4).

Recently, the organotypic culture model has been optimized by several groups. Green et al. (41) describe greatest matrix reliability using porcine esophageal matrix or collagen, with better epithelial adhesion to porcine matrix and enhanced fibroblast migration into collagen. The esophageal epithelial cells and esophageal fibroblasts obtained directly from patients undergoing esophagectomy were associated with the best performance in this model (41). Techniques for the organotypic model, with specific information on developing this collagen-based matrix model with human or mouse esophageal cells and supporting fibroblasts, have been described by Rustgi and colleagues (63).

Kosoff et al. (74) used the organotypic culture system with a collagen matrix and a fibroblast feeder layer to describe growth of BE cell lines. One of their key findings was the heterogeneity of the four BE cell lines (CP-A, CP-B, CP-C, and CP-D) in their cellular phenotype. The CP-A cell line (from nondysplastic BE) formed goblet cells with cytokeratins, representative of squamous and columnar phenotypes; CP-B and CP-D cell lines (both from high-grade dysplasia) grew as a stratified epithelium, and CP-C cells (also from high-grade dysplasia) grew predominantly in a single layer. Some (CP-A and CP-D), but not all, of the epithelial cell lines invaded the matrix (74). When all-trans-retinoic acid was added to the organotypic culture, BE cells that had grown in a stratified pattern grew in thickness of one to two cells, and numbers of goblet cells were reduced in the CP-A cell line (74). Acid pulses (pH 3.5 for 1 h) did not change cytokeratin 13 or 14 expression. Time in the organotypic culture model was associated with reduced cytokeratin-8 (KRT8), and cytokeratin-19 (KRT19) remained elevated over time, regardless of acid exposure (74).

Organotypic models can be used to evaluate interactions between the epithelium and stroma, and cells can be exposed to acid, bile, and other agents to assess changes in morphology, invasiveness, and gene expression. However, these models are only as strong as the cells used (and the information known about those cell lines). Interpretation of results from organotypic models should be mindful of the potential for heterogeneity in cell lines used to initiate the model, and cells should be carefully characterized over time whenever possible to make relationships to human models as relevant as possible.

**Denuded trachea model.** As an alternative to the organotypic culture system, denuded trachea may be used to grow epithelial cells, as reported in 1982 by Klein-Szanto et al. (70). Wang et al. (129) further developed this denuded trachea model, allowing growth of different esophageal cell types, including epithelium and stroma. The goal has been to induce squamous epithelial cells to transdifferentiate to form BE. In this model, genetic manipulation of the donor mouse with overexpression of sonic hedgehog in the epithelium and induction of bone morphogenetic protein 4 in the stroma, lead to expression of cytokeratins 8–18 (markers of columnar epithelium) and SOX9 in the epithelium (129). The authors reported the suggestion of a columnar epithelial phenotype, although a frank BE phenotype was not generated (129).

With advances in creation of cell culture and coculture techniques, more is possible in an in vitro environment. It is conceivable that, with the correct combination of cells and environmental factors, an experimental system that mimics human esophagus, including the potential for metaplasia, can be generated. Then exposure of the cells to environmental factors associated with BE and EAC may be possible. In vitro models are limited by the various growth factors and special conditions needed to grow these cells. Esophageal cells in typical culture do not tolerate medium with pH <4.0, nor do they tolerate exposure to bile; thus, pulsed treatments are often used, and this may mimic gastroesophageal reflux disease. For bile salts, many groups have used deoxycholic acid, which is not normally found in the upper gastrointestinal tract (43, 52, 57, 82). Kosoff et al. (74) used the organotypic model to study BE cells cultured with esophageal fibroblasts. However, cell culture environments may differ substantially from the environment that is actually present in vivo. Cell culture can be useful for studying gene regulation in a controlled environment, but histological metaplasia cannot be observed using standard culture techniques.

**Animal Models**

Because of the limitations of cell culture systems and the need for models of clinical risk factors and exposures, several animal models have been developed for the study of BE and EAC, but a clearly superior model has not emerged. In humans, most EAC is closely linked with BE, and a traditional sequence of nondysplastic BE to low-grade dysplasia to high-grade dysplasia is thought to precede development of EAC, as shown in Fig. 1.

The primary animal models that have been developed to study BE include mouse and rat models. Mice and rats do not develop BE under normal conditions. In addition to the lack of a natural phenotype, the major limitation of the mouse and rat models is that they differ from humans in basic biology of the esophagus (see below). In addition to rats and mice, other animals include dogs, opossums, guinea pigs, baboons, and pigs.

To induce columnar epithelium in the esophagus of mice and rats, surgery is generally required, although Barrett’s epithelium has been rarely observed with carcinogen exposure after development of esophagitis (140). A few genetic models have been attempted, with the goal of inducing epithelium with characteristics in common with BE; yet these attempts have generally been unsuccessful. In the following section, the different model organisms for BE and the genetic studies performed in these experimental models are described.

**Rats.** Surgical operations, such as esophagoduodenostomy (25) and esophagojejunos stomy (99), which result in massive bile exposure to the rodent esophagus, are more feasible in rats than in mice, given their size. Survival after these procedures is reasonable. In a study of 100 rats subjected to esophagojejunostomy, 86% survived the surgery (104).

Rats develop cancers after esophagoduodenostomy and esophagojejunos tymostomy, although these are not always the adenocarcinomas typically seen in human progression of BE. After esophagojejunoscopy, at 8 mo of age, 62% of animals
developed cancer compared with 26% in an ursodiol-aspirin intervention arm (104). In the esophagojejunostomy model, typically about half of the cancers that developed were adenocarcinomas (104). Clark et al. (25) demonstrated that a rat esophagoduodenostomy model induced papillomatosis and hyperkeratosis in 97% and BE in 10% of the animals. Intraperitoneal injection of methyl-N-amylnitrosamine (25 mg/kg) weekly for 4 wk resulted in columnar metaplasia in 13% and cancer in 57% of the rats (42% of these were EAC, and the rest were squamous cell cancers) (25).

A key potential advantage of an animal model of BE and carcinogenesis is the ability to evaluate clinical and environmental risk factors in a controlled setting. The major risk factors associated with BE are listed in Table 2, and accepted risk factors include male sex, >50 yr of age, white race, central obesity, long-standing gastroesophageal reflux disease, and hiatal hernia (114). Smoking has increasingly been implicated as a risk factor associated with BE (29) and progression to dysplasia and cancer (27).

As an example of the power of animal models in the evaluation of clinical risk factors that pertain to humans, introduction of a high-fat diet alone in rats did not change cancer incidence after esophagoduodenostomy, but in the presence of methyl-N-amylnitrosamine and a high-fat diet, cancer rates increased from 42–55% to 83% (25). In rats undergoing esophagastroduodenostomy, addition of iron (4 mg·kg\(^{-1}\)·wk\(^{-1}\) ip) increased the incidence of columnar epithelium in the esophagus from 53.5% to 78% and increased the incidence of mucinous adenocarcinoma of the esophagus from 25.6% to 53.7% (23). However, there are no epidemiological data to support a positive risk association between iron and BE or EAC in humans, and one study demonstrated a negative association of serum ferritin, toenail iron stores, and lifestyle questions on iron intake with BE (90). The surgical rat model also forms multilayered epithelium, which may be an intermediate in the development of BE (20). Cyclooxygenase-2 (COX2) inhibitors decrease the rate of cancer development in rats that have undergone esophagogastricomy (14), suggesting a role for prostaglandins in development of BE.

Without significant surgical or chemical exposures, generation of BE remains challenging in rat models for several reasons. The rat esophagus has extensively keratinized squamous epithelium compared with the nonkeratinized squamous epithelium in humans, and rat esophagus lacks the submucosal glands found in humans (22). Because of the acid neutralization that normally occurs in the small bowel, the reflux created in esophagoduodenostomy and esophagojejunostomy is less acidic than that in a model of reflux from the stomach, and the concentration of bile in the refluxate is presumed to be much greater than that in human gastroesophageal reflux disease. While BE can be generated with some effort in rat and mouse surgical models, even in the absence of submucosal glands, this does not exclude the possibility that, in humans, BE is derived from esophageal submucosal glands. Whether animals with submucosal glands will develop BE more readily than rats remains to be answered.

**Mice.** The very clear and important advantage of mouse models for the study of BE and EAC is the potential use of genetic manipulation. As a model organism for BE, mice are
similar to rats in the absence of esophageal submucosal glands. The squamous epithelium in mice, as in humans, is p63-positive, although in mice (and rats), the squamocolumnar junction extends to the midstomach (133). In wild-type mice and rats, columnar epithelium does not develop within the forestomach, although constant exposure to stomach acid, an additional difference between mice and humans. Mice are smaller than rats, and surgical approaches can be difficult for this reason. As in the rat models, in mouse models of BE, columnar epithelium is created only in the presence of highly destructive concentrations of bile salts at more neutral pH. However, the benefit of the mouse’s clearly described and well-characterized genome, combined with the availability of knockout, knockdown, and knockin models, makes this an attractive organism to investigate. Some of the studies that have been performed in mice to evaluate esophageal epithelial biology are reviewed in this section.

A dietary model of BE that uses zinc deficiency and inclusion of deoxycholic acid in feed has been developed in male mice (43). After 88–152 days, 63% of C57BL/6 mice developed BE-like lesions preceded by esophagitis. The BE-like lesions demonstrated goblet cells and MUC2 expression (43).

The surgical models of esophagoduodenostomy and esophagoj stoolnostomy can be very difficult to perform in these small animals, with mortality of 20–50% (22, 34, 140). The first study describing esophagojejunostomy in mice, rather than rats, included three groups: 37 mice underwent esophagojejunostomy alone, 39 underwent esophagojejunostomy and were exposed to the carcinogen N-methyl-N-benzyltrimorosamine (MBN), and 32 mice were exposed to only MBN. After 19 wk, survival was as follows: 89% of those with esophagojejunostomy alone, 90% of those with MBN exposure via weekly injection (2.5 mg/kg ip) starting 1 wk after esophagojejunostomy, and 100% of those with only MBN exposure (140). Of the mice that underwent surgery alone, 42% developed BE and 12% developed cancer. In the group subjected to surgery and exposed to MBN, 20% developed BE alone and 54% developed cancer. In the mice exposed to carcinogen alone, 13% developed BE and 47% developed cancer. As seen in the rat models, not all the cancers were adenocarcinomas: esophagojejunostomy alone was associated with development of squamous cell cancer of the esophagus in about half of the mice (140).

In response to the technical difficulty associated with esophagojejunostomy in mice, a model has been reported in which esophagoj eunal anastomosis was created through the use of neodymium micromagnets (34). In 80 animals, they reported 5% mortality (34). Development of severe esophagitis and columnar metaplasia began at 12 wk. However, no goblet cells were demonstrated in the area of metaplasia, and it remains possible that the columnar epithelium comes directly from progenitor cells in the intestine that is now in contact with the esophagus.

**Genetic models in mice.** Several genes and pathways have been implicated in the development of BE and EAC. While a detailed discussion of the genes associated with BE and EAC is beyond the scope of this review, Table 3 lists several of the genes that have been associated with BE and EAC. Classically, BE has been associated with loss of gene expression linked to squamous differentiation, such as SRY (sex-determining region Y) box 2 (SOX2) and p63 (20, 44) and gain of gene expression associated with columnar differentiation, such as caudal-type homeobox 1 (CDX1) and caudal-type homeobox 2 (CDX2) (50, 111, 127, 138). Gene expression in humans may be altered through allelic loss, point mutations, or epigenetic alterations. Pathways such as transforming growth factor-β (TGF-β), Wnt, nuclear factor κ light chain enhancer of activated B cells (NF-κB), and hedgehog have also been linked to BE and EAC (26, 51, 84, 91, 107, 141). Overall, inherited gene mutations have been difficult to associate with development of EAC. A few glutathione transferases have been associated with EAC (61, 88), particularly in the setting of tobacco smoke exposure (15).

Using genetic manipulations, several groups have attempted to identify a mouse model of BE in mice that mimics human histology. Individually targeting several different key genes thought to be implicated in the development of human BE has not reproduced the BE phenotype in mice.

As described by Que et al. (102), mice with SOX2 knockout developed a phenotype that included a ciliated columnar epithelium with decreased p63-positive basal cells and decreased cytokeratin 14 (KRT14) expression (normally found in squamous esophageal basal cells). Developmentally, this phenotype represents a failure of normal squamous development. This model was not histologically identical to human BE with columnar epithelium without goblet cells. The authors concluded that SOX2 maintains the proliferation of cells from the basal layer, promoting differentiation and development of the stratified epithelium in mice (102). The SOX2-deficient mice developed other tracheoesophageal abnormalities that affect viability (102).

Genetic models in mice can be combined with esophageal exposures and surgical models to better study the pathogenesis of BE. In a separate study, in p53 knockout mice, zinc deficiency combined with exposure to the carcinogen N-nitrosylmethylbenzylamine has been reported to promote “glandular metaplasia” resembling BE in 19% of mice (38), but this is not a widely used model.

Wang et al. (129) used a mouse model of reflux with the mouse esophagojejunostomy model, as described by Sui et al. (117), to induce a Barrett’s-like epithelium in mice with a Pch1-LacZ reporter to evaluate hedgehog signaling. At 20–35 wk following esophagojejunostomy, hedgehog signaling was increased, but no BE appeared; BE mucosa did not appear until 39 wk (129). One important observation from this study was that hedgehog activity was found in the stroma, near the BE epithelium, indicating stromal response to esophageal inflammation (129).

Hao et al. (47) describe a different experimental model of genetic manipulation combined with a surgical model. When wild-type, p53 transgenic mice and cyclin-dependent kinase inhibitor 2A (CDKN2A)/INK4a/Arf+/− mice underwent esophagogastrudodenostomy, none of the mice of A/J strain developed EAC (47). Only 5% of wild-type and p53 transgenic mice developed esophageal metaplasia after surgery (47). Of the CDKN2A+/− mice, 7.1% developed squamous cell cancers (47). One interesting question posed by these authors was the role of genetic background in the mice and cancer development, given that Swiss-Weber mice were used for the original experiments with higher rates of EAC (47).

Esophageal-specific cytokeratin promoters can drive targeted gene expression or deletion of genes of interest. This can be of importance when a gene is essential to viability and knockout may...
## Table 3. Genes and pathways implicated in development of BE and EAC

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Name/Description</th>
<th>Location in Human</th>
<th>Animal Models</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Increased in BE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACTA2</td>
<td>Actin, α2, smooth muscle, aorta (α-SMA) A protein found in smooth muscle, and a marker of myofibroblasts; it has a role in cell structure, contraction, and motility.</td>
<td>Increased in subepithelial myofibroblasts in BE compared with normal and increased in dysplasia (92)</td>
<td>None reported for esophagus</td>
</tr>
<tr>
<td>BMP4</td>
<td>Bone morphogenetic protein 4 Protein that is a member of the TGF-β superfamily</td>
<td>Increased expression in esophageal stroma in BE (16, 129) In vitro, acid and bile may increase BMP4 in epithelial cells (144)</td>
<td>Increased expression in mouse model of IL-1β overexpression with columnar phenotype (100)</td>
</tr>
<tr>
<td>CDX1</td>
<td>Caudal-type homebox 1 Transcription factor</td>
<td>Not reported in normal esophagus; increased in BE and present in intestine (111, 138)</td>
<td>Rat model; found in squamous and columnar in rat surgical model (67)</td>
</tr>
<tr>
<td>CDX2</td>
<td>Caudal-type homebox 2 Transcription factor</td>
<td>Increased in BE (36) and increased mRNA with esophagitis; may decrease with dysplasia and in EAC (50, 127)</td>
<td>Mouse model of CDX2 expression (72)</td>
</tr>
<tr>
<td>COX2</td>
<td>Cyclooxygenase-2 Enzyme (prostaglandin synthesis)</td>
<td>Increased in human squamous esophagus with reflux (46) Increased in BE epithelium and EAC (64, 86, 137)</td>
<td>Rat surgical model increased COX2 in inflammatory cells in stroma and in basal and suprabasal esophageal cells (19)</td>
</tr>
<tr>
<td>CCND1</td>
<td>Cyclin D1 Cell cycle protein G1-to-S transition</td>
<td>Epithelial expression in BE with nuclear staining (6, 8, 124)</td>
<td>Mouse model of cyclin D1 transgenic mice with increased epithelial proliferation and dysplasia (87)</td>
</tr>
<tr>
<td>COL5A2</td>
<td>Collagen, type V, α2 Fibrillar collagen molecule (stromal)</td>
<td>Increased in stroma of BE and EAC by gene expression and in situ hybridization for RNA (48)</td>
<td>None reported</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal growth factor receptor Transmembrane glycoprotein; a protein kinase</td>
<td>Step-wise increased expression in some BE, in dysplasia, and EAC (31)</td>
<td>EGFR-overexpressing mice with increased basal proliferation in esophageal basal and suprabasal layers (5)</td>
</tr>
<tr>
<td>IL1B</td>
<td>Cytokine produced by activated macrophages (activated by caspase-1 and IL-1β induces COX2)</td>
<td>Mucosal IL-1β expression correlates with local inflammation in esophagogastric cancer (33)</td>
<td>Overexpression of IL-1β in mice resulted in columnar metaplasia in forestomach (100)</td>
</tr>
<tr>
<td>KLF4</td>
<td>Knüppel-like factor 4 (gut) Zinc finger-containing transcription factor</td>
<td>Increased in BE (68) Decreased expression in squamous cell cancers of the esophagus (132) May be associated with DNA repair (142)</td>
<td>Rat surgical model demonstrated increase in columnar epithelium (68)</td>
</tr>
<tr>
<td>LGR5</td>
<td>Leucine-rich repeat-containing G protein-coupled receptor 5 Protein that encodes a G-coupled protein receptor; intestinal stem cell marker</td>
<td>LGR5 found in most cases of BE and nearly all EAC (11) LGR5 found in EAC with and without BE surrounding the cancer (128)</td>
<td>May be associated with progenitor for columnar metaplasia found in IL-1β model (100)</td>
</tr>
<tr>
<td>POSTN</td>
<td>Periostin, osteoblast-specific factor Protein has cell adhesion roles</td>
<td>Increased in stroma of BE and EAC by gene expression and in situ hybridization for RNA (48)</td>
<td>None reported</td>
</tr>
<tr>
<td>SHH</td>
<td>Sonic hedgehog Protein ligand that participates in morphogenic signaling pathway</td>
<td>Increased in columnar metaplasia (nongoblet) (141) Increased in BE epithelium and EAC(129)</td>
<td>SHH transgenic mouse epithelium grown in culture and associated with increased BMP in stroma and SOX9 in epithelium (129)</td>
</tr>
<tr>
<td>SOX9</td>
<td>SRY (sex-determining region Y) box 9 HMG box-class DNA-binding proteins</td>
<td>Increased in BE and EAC (129) (epithelial SOX9 induced by BMP4 in stroma) Present in epithelium; increased with HGD in upper crypts (32)</td>
<td>None reported</td>
</tr>
<tr>
<td><strong>Decreased in BE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDH1</td>
<td>E-cadherin Calcium-dependent cell-cell adhesion glycoprotein</td>
<td>Decreased /abnormal E-cadherin-β-catenin expression in BE and EAC (118, 134); E-cadherin cleaved in esophageal epithelium of patients with GERD (60)</td>
<td>E-cadherin knockout mouse model (60)</td>
</tr>
<tr>
<td>CDKN2A (p16)</td>
<td>Cyclin-dependent kinase inhibitor 2A Tumor suppressor cell cycle protein, G1 control</td>
<td>p16 abnormalities increase with histological grade (97)</td>
<td>p16 haplo-insufficient mice have been used in a mouse model of BE (101)</td>
</tr>
<tr>
<td>CDKN1A</td>
<td>Cyclin-dependent kinase inhibitor 1</td>
<td></td>
<td>As a link to clinical risk factors, p16 deficiency has been found in mouse model of atherosclerosis (75)</td>
</tr>
</tbody>
</table>
be embryonically lethal. Patterns of cytokeratin expression are noted in Table 4. Several cytokeratin promoters, including cytokeratin 5 (KRT5) (21), have been used to drive esophageal expression, but reports using the KRT14 promoter, as developed by Vasioukhin et al. (126), are by far the most common in the literature. While classically described as a promoter in the epithelial types, at the juncture of KRT5/KRT14 and KRT8/KRT18, this promoter results in increased PTCH and LDL receptor-related protein receptors; binding of Wnt ligand to these receptors prevents degradation of ß-catenin (CTNNB1), a key transcription factor when localized to the nucleus (136); ß-catenin also interacts with E-cadherin at the adherens junction; core proteins encoded by AXIN1, APC, glycogen synthetase kinase 3 (TPPK), and casein kinase 1 (CSNK1A1) act together to phosphorylate ß-catenin for ubiquitinization (69); if it escapes degradation in the nucleus, ß-catenin acts through T cell factor/lymphoid enhancer factor (TCF/LEF) and results in increased myc, matrix metalloproteinase 7, and cyclin D1 (26). It is believed that Notch signaling can direct development along a specific path of differentiation (73); Notch receptors (Notch 1-4 in mammals) are activated by ligands such as Jagged (JAG1) to release the Notch intracellular domain (NCID) and activate genes such as Hes1, Hes5, and Hes7, as well as Hey1, Hey2, and HeyL (65). Notch signaling regulates intestinal epithelial cell differentiation and inhibits the secretory GI cell phenotype (125) and is the Hh receptor to which Hh ligand binds, releasing SMO and activating Gli transcription factors (103). SHH and PTCH1 were present in columnar epithelium, as was BMP4; patients with more deoxycholic acid had increased PTCH and BMP4 (141). Constitutive Notch signaling in EAC with increased Hes1 and Jagged1 (84) overexpression with columnar phenotype; Notch inhibition in IL-1ß mouse model resulted in more goblet-like cells (100).

EAC, esophageal adenocarcinoma; APC, adenomatous polyposis; HGD, high-grade dysplasia; HMG, high-mobility group; SMO, smoothened; GI, gastrointestinal.
associated with increased goblet-like cells, although classic goblet cells were not observed in this model (100).

Another potentially paradigm-shifting model was the recently reported mouse model of BE by Wang et al. (133) in p63-deficient mice. These animals develop to full term, but the p63 deletion is lethal. As in the SOX2 knockout model, normal squamous development is disrupted by p63 deletion. In the embryonic p63 null animals, the squamocolumnar junction is shifted, and a columnar epithelium is found (at embryonic day 18) in the proximal stomach, rather than the normal squamous epithelium, resembling BE, although without the characteristic goblet cells (133). Generally, gene expression patterns of this metaplastic stomach in these animals is similar to that found in human BE, with notable exceptions, such as CDX2, which is not expressed in the p63 null metaplasia and is found in human BE (133).

Other Animals

Several other animals, including dogs, pigs, guinea pigs, and opossum, have been considered as models for the study of BE. All these animals have submucosal esophageal glands as humans do, and many lack the degree of keratinization seen in mice and rats, and thus they may provide more suitable models for the study of development of BE and EAC in animals (2, 22, 80).

In a dog (Canis familiaris), columnar metaplasia has been shown to arise directly from cells intrinsic to the esophagus (as migration from the stomach was blocked) (39). Mucosal stripping in dogs combined with reflux results in columnar metaplasia, particularly around the submucosal glands, but goblet cells are not typically noted in these experiments (39, 79). Various surgical approaches, including cardectomy and total gastrectomy with esophagojejunostomy, have been used to induce reflux in dogs (66). In these models, BE with goblet cells develops after 18–39 mo, and high-grade dysplasia and EAC generally develop after >60 mo (66). A major disadvantage is the length of time needed for these experiments. An advantage is that the type of EAC is closer to the type developed in humans (a glandular type, rather than the mucinous type, which develops in rats and mice) (22, 66).

The opossum (Didelphidaeae) has been evaluated for its submucosal glands and has been used to study bicarbonate secretion in the esophagus; these experiments demonstrated that an important component of acid neutralization in the opossum comes from the submucosal glands, as is seen in humans (1, 45). Guinea pigs (Cavia porcellus) have been used to study lipid metabolism, obesity, and immunology, given their similarities to humans in these metabolic areas (30, 89, 112). Published reports of the guinea pig as a model organism for BE are lacking (PubMed search on 1 Nov 2011), despite the frequent use of guinea pigs in research and the presence of submucosal glands in these animals.

Pigs (Sus scrofa) are close to humans biologically, but significant resources are required to breed and maintain these animals. Pigs have the submucosal glands found in humans, and these glands are similar to submandibular salivary glands and bronchial glands (2). The glands are large enough to be dissected from the pig esophagus and grown in culture (2). Closely associated fibroblasts have been noted when these glands are grown in culture. The serous demilunes in the pig esophageal submucosal glands stain for lysozyme (2). The pig
submucosal glands have been characterized by their cytokeratin expression using immunolocalization (2). While pigs have been used frequently to test new endoscopic techniques for ablation of BE, published reports of surgical models or spontaneous models of BE forming in the pigs are lacking (PubMed search on 1 Nov 2011). A pig model is in development for the study of BE in a joint effort between investigators (including X. L. Chen and R. C. Orlando) at North Carolina Central University, the University of North Carolina at Chapel Hill, and North Carolina State University.

One key observation is that pigs differ from mice, and transgenic models may yield different phenotypes in these two animals. For example, using the KRT5 promoter to evaluate sonic hedgehog-Gli signaling in skin, changes were noted in pigs (generally more similar to humans) that were not observed in mice (83).

Baboons (Papio) exhibit behavior of chewing regurgitated food, and they spontaneously develop “mucus gland metaplasia” in the esophagus, similar to BE (92% of the 50 samples assessed) (106). However, working with primates is logistically challenging, and the baboon genome is not complete.

Given the recent advances with genomic sequencing of many organisms, the guinea pig, opossum, dog, and pig genomes have been sequenced and are available using the Ensembl Genome browser. Thus genetic studies may become more feasible in these animals.

One potential benefit of a stronger animal model of BE and EAC would be biomarker development, which might advance our ability to screen for BE or EAC in humans. Such a longitudinal model might help us predict which patients are at highest risk of cancer development, particularly given the ablative strategies now possible. Identification of BE depends on the presence of mucus-producing goblet cells in the setting of columnar metaplasia. Only one marker, trefoil factor 3 (TFF3), has been adopted for clinical use as a biomarker for noninvasive BE screening (76). The American Gastroenterological Association does not recommend any specific biomarkers as histological markers of BE, given the lack of evaluation in prospective controlled clinical trials (114). On the basis of the review of human gene expression data with correlation with immunohistochemistry by Wang et al. (131), several markers are found exclusively in BE mucosa, and these could be studied in future experimental models of BE (131).

The Future

Ideally, a model system would be developed for the study of BE that would allow histological, genomic, and mechanistic evaluation of the changes that occur in the original metaplasia and in the development of dysplasia and cancer. Animal models offer the advantage of in vivo assessment of metaplasia and dysplasia, but the lack of spontaneous development of BE has been a limitation.

Surgical models of esophageal injury via esophagoduodenostomy or esophagojejunostomy have several limitations. There is technical difficulty associated with these procedures, and mortality rates are high. Moreover, the postoperative state created does not clearly mimic the high-acid, low-bile state seen in most humans. Only about half of the tumors that develop in mice and rats after such operations are adenocarcinomas.

Consideration of the origin of the progenitor cell in BE is important. While the basal squamous cells may be the source of normal progenitors in the squamous esophagus, it remains unclear if these are also the progenitors for the Barrett’s epithelium. An animal model with submucosal glands such as is found in the pig, guinea pig, and opossum more closely mimics the human esophagus with the presence of these glands. It is possible that, in animals with submucosal glands, metaplasia can be induced through new methods of esophageal injury or exposure that have not yet been possible in the mouse or rat. Label-retention studies or lineage-tracing experiments in an animal model of BE would be quite helpful at understanding cellular origin of the metaplasia.

The American Gastroenterological Association recently recommended screening for patients with clinical risk factors for BE, such as male sex, white race, chronic reflux, and obesity (114). In addition to these risk factors, smoking has recently been identified as a risk factor for BE (54, 116), EAC (28), and recurrence of BE after ablation (7). Alcohol use has not been associated with increased risk of BE (121). Ideally, an animal model would allow evaluation of the development of BE in the setting of known clinical risk factors for BE to assess changes in cell signaling and pathway activation associated with the development of Barrett’s metaplasia and EAC.

On a molecular level, an animal model of BE and EAC with potential for genetic manipulation, such as the currently available mouse models, would be ideal. To understand the molecular signals that contribute to the development of BE and EAC, an animal model of BE would need to allow for evaluation of cross talk between stroma and overlying epithelium. As the genomes of various animals, including the guinea pig, pig, dog, and opossum, have been published, more complex evaluations of BE and development of EAC may be possible in animals other than the mouse.

Leveraging advances in genomics and genetics with classical understanding of cellular biology and physiology may help us respond to the many unanswered questions about the development of BE and EAC. As the incidence of EAC continues to climb (13), using complex experimental models to understand the interplay between genetic risk, environmental exposure, and potential preventive strategies will be critically important.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

K.S.G., R.C.O., and X.L.C. are responsible for conception and design of the research; K.S.G. prepared the figures; K.S.G. drafted the manuscript; K.S.G., R.C.O., and X.L.C. edited and revised the manuscript; K.S.G., R.C.O., and X.L.C. approved the final version of the manuscript.

REFERENCES


