Acid, Bile, and CDX: the ABCs of making Barrett’s metaplasia

Rhonda F. Souza,1,2 Kumar Krishnan,1 and Stuart Jon Spechler1

1Department of Medicine, Veterans Affairs North Texas Health Care System and the University of Texas Southwestern Medical School and 2Harold C. Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas

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IT HAS BEEN ESTIMATED THAT 20–40% of adult Americans have gastroesophageal reflux disease (GERD), a chronic condition that can cause inflammation in the esophageal squamous epithelium (i.e., reflux esophagitis) (42). In most patients, squamous epithelium damaged by reflux esophagitis heals with the regeneration of more squamous cells. In a substantial minority of cases, however, reflux esophagitis heals through a metaplastic process in which intestinal-type columnar cells replace reflux-damaged squamous ones (67). This condition, called Barrett’s esophagus, is a major risk factor for esophageal adenocarcinoma, a deadly malignancy whose frequency has been rising dramatically and whose mortality rate still exceeds 80% (53). In the United States, the incidence of esophageal adenocarcinoma has increased by more than sixfold in the past three decades, a phenomenon that has sparked intense interest in the molecular biology of the precursor lesion, Barrett’s metaplasia (58). An understanding of the early molecular events involved in the pathogenesis of Barrett’s esophagus might facilitate the development of therapies that could prevent esophageal adenocarcinoma. The precise molecular events mediating the transformation of squamous epithelium into Barrett’s metaplasia are poorly understood, but recent studies suggest key roles for certain developmental pathways, morphogenetic factors, and homeobox (HOX) genes, which encode for transcription factors that regulate the development of intestinal epithelial cells (7, 19, 46, 50, 68, 74). This report reviews the molecular events triggered by GERD that may be involved in the pathogenesis of Barrett’s metaplasia. Throughout this review, the Caudal HOX genes for humans are designated all in capital letters as CDX, whereas those nonhuman genes are designated Cdx.

Barrett’s Esophagus: A Metaplastic Response

One way in which tissues respond to chronic inflammation is through metaplasia, the process in which one adult cell type replaces another. In theory, metaplasia can result from the abnormal differentiation of stem cells or from the transdifferentiation of one mature cell type into another type of mature cell (16). In many cases, metaplasia appears to be a protective...
response because the metaplastic tissue is more resistant than the native tissue to the noxious agents causing the chronic inflammation. For example, chronic gastritis due to infection with *Helicobacter pylori* (*H. pylori*) results in intestinal metaplasia, which is less susceptible to *H. pylori* infection than the normal gastric epithelium. In Barrett’s esophagus, reflux-damaged squamous epithelium is replaced by an intestinal-type epithelium, called specialized intestinal metaplasia, that appears to be more resistant to acid-peptic damage.

Early investigators proposed that Barrett’s esophagus resulted when columnar cells of the proximal stomach migrated up the esophagus to reconstitute the reflux-damaged squamous epithelium (9). This migration hypothesis is no longer favored because it does not account for the intestinal-type cells that are characteristic of Barrett’s metaplasia and because, in certain animal models of Barrett’s esophagus, columnar epithelium can be found in parts of the esophagus that are not contiguous with the stomach (20). For the past three decades, the prevailing hypothesis has been that Barrett’s metaplasia results when reflux esophagitis damages the superficial layers of the esophageal squamous epithelium while stimulating the abnormal differentiation of stem cells in the basal layers (32, 54, 64).

Some have proposed that the stem cells that give rise to specialized intestinal metaplasia are located in the ducts of the submucosal esophageal mucosal glands rather than in the squamous epithelium itself (21). In either case, the progenitor cells for Barrett’s metaplasia have been assumed to originate within the esophagus itself.

A recent study has suggested the possibility that stem cells from the bone marrow might contribute to the development of Barrett’s esophagus (63). In that study, female rats were given a lethal dose of radiation to destroy their native bone marrow cells followed by a bone marrow transplant from male rat donors. The transplant recipients then had an esophageojunostomy, which resulted in severe esophagitis and intestinal metaplasia similar to that seen in patients with Barrett’s esophagus. Eight weeks after esophageojunostomy, nuclear staining for Y chromosome was found both in squamous cells and in metaplastic columnar cells in the esophagus of the female rats. These observations show that multipotent, adult progenitor cells of bone marrow origin contribute to esophageal regeneration and metaplasia in this rat model of reflux esophagitis and Barrett’s esophagus. This study raises the possibility that the progenitor cell for Barrett’s esophagus might be a circulating, bone marrow-derived stem cell. It is not clear that humans have circulating stem cells that can home in to areas of injury to repair damaged tissue, however, and the applicability of these findings to human Barrett’s esophagus has not been established.

In general, metaplasias involve the transformation of adult tissue into a type that was present in the organ during embryological development (73). In the human embryo, a single, hollow endodermal tube gives rise to both the respiratory tract and the esophagus. The epithelium of this endodermal tube comprises two or three cell layers, which appear to arise from a common progenitor cell that expresses p63, a homologue of p53 (13). As the endodermal tube matures and forms the esophagus, its p63+ progenitor cells differentiate into ciliated, columnar cells that do not express p63. At about the seventeenth week of development, squamous epithelium first appears in the mid-esophagus, from where it extends both proximally and distally to replace the ciliated, columnar epithelium (24, 33). Cells in the basal layer of the stratified squamous epithelium exhibit strong expression of p63, whereas the fully differentiated squamous cells in the more superficial layers show no p63 staining (13). These data suggest that an embryonic esophageal stem cell that expresses p63 has the capacity to give rise to both columnar and squamous epithelial cells (13). However, Daniely et al. (13) and Glickman et al. (22) found no immunostaining for p63 in Barrett’s epithelium. The absence of p63 immunostaining in Barrett’s metaplasia does not help to determine whether the metaplasia developed through the transdifferentiation of mature squamous cells or through the abnormal differentiation of stem cells from the esophagus or the bone marrow. However, the complete absence of p63 in Barrett’s metaplasia does suggest that the stem cells that maintain this abnormal epithelium differ from the p63+ progenitor cells of the embryonic esophagus and the normal, adult squamous esophagus.

**The Role of HOX Genes in Embryological Development**

Homeosis is a word of Greek origin meaning a shift in structural development. A homeotic gene is one that produces a major shift in the developmental fate of an organ. Metaplasias result from changes in the expression of homeotic genes (16, 60, 73). Genes of the HOX family contain a highly conserved DNA sequence called the homeobox, which encodes a DNA-binding amino acid sequence (called the homeobox domain) of certain proteins that function as transcription factors. The HOX genes play an essential role during embryological development by imparting “positional information” that results in the segmental patterning of vertebrate development (7). HOX genes are expressed in tissues and structures that arise from the ectoderm and mesoderm (e.g., dermatomes and musculoskeletal and nervous systems) (7). The closely related Para-Hox genes are responsible for determining gut patterning from the endoderm (7). The Para-Hox group comprises two distinct families of homeobox genes: 1) pancreatic duodenal homeobox 1, which is involved in early development of the pancreatic buds, β and δ cells of the pancreatic islets, and the duodenum, and 2) the Cdx genes (Cdx1, Cdx2 and Cdx4), which are homologues of Caudal, a gene found in Drosophila (3, 7, 52). Of these, only Cdx1 and Cdx2 play a role in gut development.

**The Role of Cdx Genes in the Development of Intestinal-Type Morphology**

In the human embryo between gestational weeks 8 and 10, the primitive gut develops an intestinal epithelium comprising enterocytes, goblet cells, enteroendocrine cells, and Paneth cells (25). The intestinal villi do not mature and the intestinal cells do not express their adult gene patterns until late in embryogenesis and early in the postnatal period (25). Expression of Cdx1 and Cdx2 mRNAs begins in the developing mouse intestine in utero around day 12, and this expression continues into adulthood (25). It is the expression of the Cdxs that mediates the transformation of endoderm into the simple columnar epithelium that characterizes the adult intestine (25).

The use of transgenic and knockout animals has facilitated in-depth investigation into the contribution of the Cdx genes to intestinal development. For example, mice that fail to express...
Cdx1 also fail to develop an intestinal-type epithelium (6, 43). Animals with complete loss of Cdx2 expression die in utero, but animals heterozygous for Cdx2 expression survive and develop cecal polyps, which contain a mixture of epithelial cell types including squamous cells, gastric-type foveolar cells, and parietal cells (6, 8, 10, 69). These studies suggest that normal Cdx2 expression directs endodermal cells to differentiate into phenotypes typical of the Caudal gut (e.g., small intestinal and colonic cell types), whereas low levels of Cdx2 expression cause cells to assume phenotypes typical of the anterior gut (e.g., gastric cell types) (7).

Normally, Cdx1 and Cdx2 are not expressed in the stomach. When mice are genetically engineered so that their gastric cells express Cdx1 or Cdx2, the stomach develops a metaplastic, intestinal-type epithelium containing enterocytes, goblet cells, and enteroendocrine cells (48–50, 65, 66). In addition, Paneth cells can be found in the metaplastic, intestinal-type mucosa of mice whose gastric cells express Cdx1 but not Cdx2 (49). Cdx1 and Cdx2 have been shown to regulate the expression of intestinal-type genes such as sucrase-isomaltase, MUC2, furin, and Krüppel like factor 4 (19, 25, 68). In Cdx2 transgenic mice, the intestinal-type epithelial cells develop a brush border with alkaline phosphatase activity (48). In human colorectal carcinoma cells, expression of Cdx1 or Cdx2 results in enhanced cell-to-cell adhesion, the development of a columnar cell shape, and cell polarization with surface microvilli (37). Cdx2-mediated expression of cell adhesion proteins such as E-cadherin, LI-cadherin, and claudin-2 appears to play a role both in maintaining intestinal cell morphology and polarity (25, 37).

**The Role of Cdx Genes in the Development of Intestinal-Type Function**

The data discussed above suggest that Cdx expression plays a key role in the development of typical intestinal cell morphology. Morphology does not always predict cell function, however. Important enterocyte functions include the expression of enzymes for digesting disaccharides and oligopeptides and the absorption of nutrients. In the intestinal epithelial cell (IEC)-6 intestinal cell line, Cdx2 has been shown to regulate the expression of enzymes involved in intestinal digestion and absorption such as sucrase-isomaltase, lactase-phlorizin hydrolase, calbindin-D9K, and carbonic anhydrase I (12, 15, 18, 68). In the stomachs of Cdx2 transgenic mice, the luminal surfaces of metaplastic enterocytes develop microvilli that express sucrase and the peptide transporter PepT1 (50). When homogenates of normal stomach, normal small intestine, and intestinal metaplasia from the stomachs of Cdx2 transgenic mice are incubated with disaccharides, glucose (monosaccharide) levels increase in the homogenates from normal intestine and intestinal metaplasia but not in those from normal stomach (50). This shows that the intestinal metaplasia induced by Cdx2 expression maintains the ability to digest disaccharides (50). The metaplastic mucosa also retains the activity of leucine aminopeptidase, indicating that it is capable of digesting peptides (50). In one interesting series of experiments, Cdx2 transgenic mice with intestinal metaplasia in the stomach and wild-type control mice had glucose and amino acids administered directly into their stomachs, which had been ligated at the pylorus to prevent emptying into the intestine (50). Serum levels of glucose and amino acids increased in the Cdx2 transgenic animals but not in the controls, showing that the intestinal-type metaplastic mucosa in the stomach had intestinal absorptive capacity (50). Thus Cdx2 expression in the stomach results in the development of a metaplastic epithelium that has morphological and functional features typical of normal intestinal mucosa.

**Interaction of Cdx Genes with Key Developmental Signaling Pathways**

Cdx regulation is an incompletely understood process but likely involves complex interactions among key signaling pathways involved in regulating embryonic development and in maintaining the homeostasis of adult tissues. Pathways that have been proposed to play a role in the regulation of Cdx expression include Hedgehog, bone morphogenetic protein (BMP)-4, Wnt, and fibroblast growth factor (FGF) signaling cascades.

The Hedgehog signaling pathway is known to play important roles in embryonic tissue development and in the maintenance of tissue homeostasis. Hedgehog ligands bind to their receptors, which are members of the Patched (Ptc) family (34). Downstream of Ptc is GLI, which functions as the central node in the Hedgehog pathway. The stability of the GLI protein is regulated by a “destruction” complex comprising casein kinase Ia, glycogen synthase kinase-3β, and protein kinase A. In the absence of Hedgehog signaling, Ptc family receptors inhibit the Smoothened (SMO) signal transducer, which results in the formation of the destruction complex that phosphorylates GLI, thereby targeting it for ubiquitination. Ubiquitinated GLI is partially degraded into a protein that acts as a transcriptional repressor (34). When the Patched receptors are activated by binding Hedgehog ligands, SMO proteins are released to activate the STK36 serine/threonine kinase, which prevents formation of the GLI protein destruction complex (34). This allows the full-length GLI protein to translocate to the nucleus where it induces the transcription of a number of genes including BMP-4 (31, 34).

The Sonic hedgehog (Shh) member of the Hedgehog ligand family plays an important role in the development of foregut structures including the trachea and esophagus (29, 40). In the developing mouse embryo whose esophagus has separated from the trachea, Shh expression can be detected in esophageal but not in tracheal endoderm (29, 40). Ptc, the Shh receptor, is expressed by mesenchymal cells adjacent to the endodermal cells that express Shh. Thus endodermal expression of Shh has been shown to regulate mesenchymal expression of BMP-4 (29, 40). Studies in frogs suggest that this “cross-talk” between epithelial and connective tissue signaling molecules is essential for complete maturation of the gut epithelium (30).

Unlike the columnar epithelial cells of the embryonic esophagus, the squamous epithelial cells of the adult esophagus do not express Shh (76). In fetal mice, the dorsal endoderm is surrounded by mesoderm that expresses BMP-4 (59). The cells of the dorsal endoderm that give rise to the esophageal epithelium express the BMP-4 antagonist noggin, which inhibits the effects of mesodermal BMP-4 and thereby allows the endodermal cells to acquire the flatter and thinner shape characteristic of squamous cells (59). Thus it appears that the change from a columnar to squamous epithelium in the esophagus is associated with an interruption of signaling through the BMP-4.
pathway. In addition to noggin expression, it is also possible that decreased expression of Shh by esophageal epithelial cells contributes to the decline in BMP-4 pathway signaling that triggers the change from columnar to squamous cells in the esophagus. Studies in frogs have shown that the Cdx pathway can partially compensate for the loss of BMP signaling in providing positional information to the developing mesoderm (57). Given this relationship between BMP and Cdx, it is possible that a decrease in Cdx expression also may underlie the columnar-to-squamous transition of the fetal esophagus. Finally, studies in frogs suggest that the Wnt and FGF pathways also contribute to the regulation of Cdx expression in the developing endoderm and mesoderm (36, 39).

The Making of Barrett’s Metaplasia

Morphologically, the specialized intestinal metaplasia of Barrett’s esophagus resembles small intestinal mucosa with its villiform surface and crypts (2). The villi are lined by intestinal-type goblet cells and by columnar cells. Transmission electron microscopy shows that the columnar cells of this specialized epithelium differ from normal intestinal absorptive cells by lacking well-defined brush borders and by containing glycoprotein secretory granules in the apical cytoplasm. Mucin histochemical staining can reveal neutral mucins similar to those of the stomach, acid-nonsulfated mucins like those of the small intestine, and colonic-type acid-sulfated mucins in the columnar surface cells. The crypt-like glands of specialized intestinal metaplasia are lined by columnar cells and goblet cells and also may contain Paneth cells and endocrine cells. Thus Barrett’s epithelium is a form of incomplete intestinal metaplasia, with a mixture of gastric, small intestinal, and colonic features.

Metaplastic conditions like Barrett’s esophagus are associated with a high rate of cell turnover that may be stimulated by chronic inflammation (73). The continuous cycle of injury and repair that accompanies chronic inflammation predisposes to alterations in the pattern of gene expression by the epithelial cells. Metaplasias occur when such alterations affect homeotic genes, like the Cdxs, that control tissue phenotypes. Metaplasias generally involve a transformation to a tissue type that was present in the organ during embryological development, and, as discussed above, the esophagus of the human embryo is initially lined by a columnar epithelium (24, 33, 73). In the human embryo, the change from columnar to squamous epithelium is associated with a decline in levels of morphogenetic factors that regulate the expression of homeotic genes like Cdx (57, 59, 73).

If declining levels of morphogenetic factors trigger the transformation from columnar to squamous epithelium in the fetal esophagus, then increasing levels of those factors later in life might be expected to reverse that process. In support of this contention, recent studies suggest a role for increasing BMP-4 expression in the squamous-to-columnar metaplasia of Barrett’s esophagus. In a rat model of GERD and Barrett’s esophagus and in humans with those conditions, BMP-4 has been found by immunostaining in the stromal tissue underlying inflamed esophageal squamous epithelium and specialized intestinal metaplasia but not in the stroma underlying normal esophageal squamous epithelium (46). When cultures of human esophageal squamous cells are exposed to BMP-4, they begin, furthermore, to express cytokeratins typical of columnar cells, and their gene expression patterns change to resemble those of Barrett’s metaplasia (46). These data suggest that GERD may cause esophageal stromal cells to express BMP-4, which promotes the change from squamous to columnar epithelium. As noted above, studies in frogs have demonstrated an interaction between the BMP and Cdx pathways (51, 57). By inference, perhaps it is the GERD-induced BMP-4 interacting with Cdx genes that mediates the development of Barrett’s metaplasia.

CDX Gene Expression in Barrett’s Esophagus

As in the animal studies discussed above, strong expression of CDX1 and CDX2 has been found in epithelial cells of the human small and large intestine but not in the normal human esophagus and stomach (65). In one study of esophageal biopsy specimens taken from patients with Barrett’s esophagus, CDX2 expression levels were ~400 times higher in the Barrett’s metaplasia than in the normal esophageal squamous epithelium, in which the levels were almost undetectable (75). Using immunohistochemistry, CDX2 expression has been demonstrated in 100% of biopsy specimens from nondysplastic Barrett’s metaplasia, Barrett’s metaplasia with dysplasia, and Barrett’s-associated adenocarcinomas (23, 56). Similarly, expression of CDX1 mRNA and protein has been found in biopsy specimens of specialized intestinal metaplasia from patients with Barrett’s esophagus but not in biopsy specimens from the normal esophagus and stomach (17, 78).

One way in which gene expression can be regulated is through alterations in the attachment of methyl groups to the promoter region of genes (gene promoter methylation). Gene promoter methylation decreases gene expression, whereas removal of those methyl groups increases gene expression. Lack of methylation of the CDX1 gene promoter can be demonstrated in the specialized intestinal metaplasia of Barrett’s esophagus, whereas normal esophageal and gastric epithelia exhibit CDX1 promoter methylation (78). Thus loss of methylation of the CDX1 gene promoter may underlie CDX1 gene expression in Barrett’s esophagus (78). Moreover, this circumstantial evidence suggests that the expression of CDX genes may underlie the development of Barrett’s esophagus.

Data on the time course of esophageal CDX expression in the development of Barrett’s esophagus emerge from studies using animal models of GERD and Barrett’s esophagus and from studies on patients with those disorders. In the animal models, GERD causes increased Cdx2 expression in cells of the basal layer of the esophageal squamous epithelium, and this increased Cdx2 expression precedes the development of intestinal metaplasia (55, 70). In esophageal biopsy specimens from patients with Barrett’s esophagus, Vallbohmer et al. (75) found higher levels of CDX2 mRNA in specialized intestinal metaplasia than in cardiac and oxynto-cardiac epithelia, which may be the precursors of specialized intestinal metaplasia. These findings suggest that GERD may initiate the development of intestinal metaplasia by increasing Cdx2 expression in cells of the basal layer of squamous epithelium. Cdx expression by these cells then increases as they assume an intestinal phenotype.
How GERD May Induce CDX Expression That Results in Barrett’s Metaplasia

Barrett’s esophagus is a consequence of chronic GERD and, as discussed above, the expression of CDX genes appears to underlie the development of Barrett’s metaplasia. There are at least two ways in which GERD might induce CDX expression in the esophagus: 1) certain components of the refluxed gastric juice (e.g., acid, bile salts) stimulate CDX expression by esophageal epithelial cells or 2) esophageal inflammation (reflux esophagitis) stimulates CDX expression by esophageal epithelial cells.

GERD can cause acid-peptic damage to the tight junctions between esophageal squamous epithelial cells, which results in increased epithelial permeability. In a series of experiments using rabbit esophageal epithelium, for example, Tobey et al. (72) found that the normal esophagus was virtually impermeable to epidermal growth factor, a peptide with a molecular weight of 6 kDa, and to dextrans with a molecular weight of 4 kDa. In contrast, esophageal mucosa exposed to acid and pepsin became permeable to epidermal growth factor and to dextrans as large as 20 kDa (72). Thus GERD-induced epithelial damage might expose stem cells in the deep layers of the esophageal squamous epithelium to acid and other noxious molecules like bile salts (71, 72, 77) (Fig. 1). This exposure might trigger the expression of CDX and other genes that cause the stem cells to differentiate abnormally into intestinal-type cells (32).

A number of in vitro studies support this hypothesis. For example, a mixture of conjugated bile acids at neutral and acidic pHs has been found to increase the expression of CDX1 mRNA in human colon cancer cells (78). In human colon epithelial cells (32), exposure to neutral pH for 48 h also activated the CDX2 gene promoter. Increased epithelial permeability, which follows GERD, might expose the esophageal stem cells to acid and bile acids (71), which are known to increase CDX1 expression in a dose-dependent manner (28). In mouse esophageal keratinocytes, chronic exposure to acid also activates the Cdx2 gene promoter (44). In OE19 esophageal adenocarcinoma cells, exposure to deoxycholate and acid enhances the binding of transcription factors to the CDX2 promoter and thereby increases CDX2 expression (14). In HET-1A and SEG-1 cells, exposure to bile acids and acid causes CDX2 promoter demethylation, which increases CDX2 expression (41). Moreover, in response to this increased CDX-2 expression, HET-1A squamous cells begin to form crypt-like structures and exhibit upregulation of intestinal genes such as villin, sucrase-isomaltase, and MUC2 (27, 41).

There are also data to support the hypothesis that esophageal inflammation (reflux esophagitis) stimulates CDX expression by esophageal epithelial cells. In human esophageal biopsy specimens, for example, CDX2 expression has been found in inflamed esophageal squamous epithelium but not in normal, uninflamed squamous epithelium (17). Moreover, CDX2 expression can be found in inflamed esophageal squamous epithelium before intestinal markers like MUC2, sucrase-isomaltase, defensin-5, and alkaline phosphatase can be detected. This suggests that CDX2 activation is an early event in the formation of Barrett’s esophagus (17). In one study, CDX2 expression was found in esophageal squamous epithelium in 6 of 19 biopsy specimens from patients who had GERD with Barrett’s esophagus, whereas none of the 40 squamous biopsy specimens from patients who had GERD without Barrett’s esophagus exhibited CDX2 expression (47). Taken together, these studies suggest that intestinal metaplasia develops when gastroesophageal reflux induces the expression of CDX2 and other morphogenetic factors in esophageal squamous epithelial cells. There is evidence that acid reflux, bile reflux, and reflux-induced inflammation all contribute to increased CDX expression levels, but the precise contribution of each of these factors remains unclear.

Fig. 1. Schematic representation of how gastroesophageal reflux may result in Barrett’s esophagus. Gastroesophageal reflux disease causes acid-peptic damage to tight junctions between squamous cells, which causes increased permeability and dilated intercellular spaces in the squamous epithelium. This exposes undifferen-tiated cells in the basal layer to acid, bile salts, and inflammatory mediators. These factors induce those cells to express Caudal homeobox (CDX) and other morphogenetic factors, perhaps through loss of methylation that activates the gene promoters. Increased expression of CDX, bone morphogenetic protein (BMP)-4, and other morphogenetic factors by the basal cells and/or underlying stromal cells appears to mediate the expression of homeotic genes that direct the squamous-to-columnar cell metaplasia characteristic of Barrett’s esophagus. The intestinal-type cells of Barrett’s metaplasia express high levels of CDX1 and CDX2.
Maintenance of Barrett’s Metaplasia: The Stem Cell

Irrespective of how Barrett’s metaplasia develops, its persistence requires that there is continual epithelial cell renewal, a process that is effected by stem cells. When gut epithelial stem cells divide, they form another stem cell (a process called self-renewal) and a progenitor cell that is capable of more rapid proliferation than the stem cell from which it arose. The progenitor cells become more differentiated and lose their proliferative capacity as they migrate toward the luminal surface of the epithelium. As discussed above, p63 has been proposed as a marker for stem cells that give rise to the stratified squamous epithelium in the embryonic esophagus (13). The stem cell that gives rise to Barrett’s metaplasia in adults remains unknown. Given that Barrett’s epithelium is a form of incomplete intestinal metaplasia, perhaps putative markers for stem cells in the intestine, which have been described only recently, might be used to identify stem cells in Barrett’s esophagus.

Properties that define stem cells include the capacities for both proliferation and self renewal, the ability to differentiate into a variety of cell types, and the ability to regenerate tissue after it has been injured (4, 62). Investigators have exploited these unique properties to identify intestinal stem cells (62). The results of such studies have yielded information on the potential numbers and locations of intestinal stem cells, but the validity of these properties to identify stem cells and, consequently, the veracity of the study findings are disputed (4, 45, 62).

Leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5), whose gene is a downstream target of the Wnt signaling pathway, recently has been proposed as a marker for intestinal stem cells (5). In mouse intestinal epithelium, cells expressing Lgr5 are relatively few in number and are located at the base of the crypts in a location that has been proposed for stem cells (5). Using genetic marking strategies, investigators have found that Lgr5+ cells exhibit functional properties of stem cells including the maintenance of proliferative capacity and the ability to generate all of the cell lineages comprising the intestinal epithelium (4, 5).

Doublecortin and CaM kinase-like-1 (DCAMKL-1), a microtubule-associated kinase, also has been proposed as a marker for intestinal stem cells (45). DCAMKL-1-expressing cells are found at the base of the crypt and immediately above the Paneth cells, another location that has been proposed for stem cells. Moreover, DCAMKL-1+ cells exhibit stem cell functional properties including the ability to undergo active cell division during the tissue regeneration that follows radiation injury (45). Whereas Barrett’s epithelium is a form of intestinal metaplasia, it is intriguing to speculate that Lgr5 and DCAMKL-1 may be useful markers for the stem cells of Barrett’s esophagus. As of this writing, however, no data are available on this issue.

The Identification of Barrett’s Stem Cells: Clinical Implications

For years, cancers of the gastrointestinal tract have been thought to arise through the step-wise accumulation of genetic alterations that endow an epithelial cell with proliferation advantages that eventuate in autonomous growth (11, 26). Proliferation of that advantaged cell clone is believed to result in the accumulation of neoplastic cells that form solid tumors. Epithelial tumors comprise a heterogeneous population of neoplastic cells, however, and it is not clear how numerous disparate cell types develop from a single epithelial cell. Furthermore, within a malignant tumor, only a small minority of the cells are capable of sustaining the cancer and of proliferating elsewhere in the body. These observations have led to the concept that malignancies develop from a small number of cancer stem cells that perpetuate themselves through self-renewal and that give rise to a variety of more differentiated neoplastic cell types, most of which are unable to form new tumors (1, 38, 61).

If the cancer stem cell concept is correct, then tumors might be prevented or eliminated by a therapy that specifically targets cancer stem cells. Therefore, the development of reliable stem cell markers could have important clinical applications. In intestinal adenomas of APC/min mice, Lgr5 and DCAMKL-1 expression has been found in only a small fraction of adenoma cells, suggesting that Lgr5 and DCAMKL-1 might be markers for neoplastic as well as normal stem cells (5, 45). Confirmation of these findings is eagerly awaited.

Cancers in Barrett’s esophagus evolve through a “metaplasia-dysplasia-carcinoma” sequence. Perhaps it is the metaplastic Barrett’s stem cell that sustains the early growth-promoting mutations in this sequence. Alternatively, a more differentiated progenitor cell might acquire mutations that confer stem cell properties. In either case, the mutated cell could give rise to disparate cell types that would have growth advantages over their normal cell neighbors. Expansion of those advantaged clones could result in morphological changes in the tissue that the pathologist can recognize as dysplasia. Further mutations in these “dysplastic” stem cells may then give rise to adenocarcinoma cells that can invade adjacent tissues and proliferate in unnatural locations. Therefore, cancer in Barrett’s esophagus might be prevented or cured by eliminating the Barrett’s stem cells. If markers such as Lgr5 and DCAMKL-1 can identify those stem cells, then it might be possible to develop pharmacological agents or endoscopic ablative therapies that can target the stem cells specifically. Such treatments would have the potential to prevent or eliminate Barrett’s esophagus, and to cure Barrett’s cancers.

Conclusion

The CDX1 and CDX2 transcription factors direct the development of intestinal cells within the gastrointestinal tract. In the esophagus, GERD causes inflammation and increased permeability of the squamous epithelium, which exposes undifferentiated cells in the basal layer to acid, bile salts, and inflammatory mediators. These factors may induce those cells to express CDX2, perhaps through loss of methylation that activates the CDX2 promoter or through stromal production of BMP-4 or other morphogenetic factors. Increased expression of CDX2 and morphogenetic factors by the basal cells appears to mediate their differentiation into the intestinal-type cells characteristic of Barrett’s esophagus, and the resultant Barrett’s metaplasia expresses both CDX1 and CDX2. Conceivably, the measurement of CDX expression levels in esophageal squamous epithelium might be used to identify which patients with GERD are likely to develop Barrett’s esophagus. Also, continuing progress in the elucidation of intestinal stem cell
markers and the regulation of stem cell behavior will likely allow for the identification of Barrett’s stem cells. Recognition of these stem cells might provide future targets at which to direct pharmacological or endoscopic therapies to prevent the development of Barrett’s esophagus and to stem the rising incidence of esophageal adenocarcinoma.

REFERENCES


