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REVIEW

Reflux esophagitis and its role in the pathogenesis of Barrett's metaplasia

Rhonda Frances Souza[1]

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Abstract Reflux esophagitis damages the squamous epithelium that normally lines the esophagus, and promotes replacement of the damaged squamous lining by the intestinal metaplasia of Barrett's esophagus, the precursor of esophageal adenocarcinoma. Therefore, to prevent the development of Barrett's metaplasia and esophageal adenocarcinoma, the pathogenesis of reflux esophagitis must be understood. We have reported that reflux esophagitis, both in a rat model and in humans, develops as a cytokine-mediated inflammatory injury (i.e., cytokine sizzle), not as a caustic chemical injury (i.e., acid burn), as traditionally has been assumed. Moreover, reflux induces activation of hypoxia inducible factor (HIF)-2α, which enhances the transcriptional activity of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) causing increases in pro-inflammatory cytokines and in migration of T lymphocytes, an underlying molecular mechanism for this cytokine-mediated injury. In some individuals, reflux esophagitis heals with Barrett's metaplasia. A number of possibilities exist for the origin of the progenitor cells that give rise to this intestinal metaplasia including those of the esophagus, the proximal stomach, or the bone marrow. However, intestinal cells are not normally found in the esophagus, the stomach, or the bone marrow. Thus, the development of Barrett's intestinal metaplasia must involve some molecular reprogramming of key developmental transcription factors within the progenitor cell, a process termed transcommitment, which may be initiated by the noxious components of the gastric refluxate. This review will highlight recent studies on the pathogenesis of reflux esophagitis and on reflux-related molecular reprogramming of esophageal squamous epithelial cells in the pathogenesis of Barrett's metaplasia.

Keywords Barrett's esophagus · Cytokine · NF-κB · Cdx2 · Squamous cells

Introduction

Gastroesophageal reflux disease (GERD) is widely regarded as the main cause of esophageal inflammation because the reflux of acid, bile salts, and other noxious agents contained in refluxed gastric juice result in reflux esophagitis [1]. Complications of reflux esophagitis include esophageal ulceration, stricture formation, and the development of Barrett's esophagus, a condition which predisposes to esophageal adenocarcinoma [2]. In the United States, GERD is extremely common, with over 20% of adult Americans having heartburn and/or regurgitation at least once per week. In Japan, the prevalence of GERD symptoms has been increasing over the past two decades [3]. During the 1990s, 10.3% of Japanese patients being seen for routine follow-up had GERD symptoms whereas the rate of these symptoms increased to 18.9% during 2000-2010 [3]. Barrett's esophagus is one of the serious complications of reflux esophagus because of its increased risk of progression to esophageal adenocarcinoma. In the United States, approximately 5.6% of adults have long-segment (≥3 cm of columnar mucosa with goblet cells) Barrett's esophagus and 10-15% have shorter segments (<3 cm) of disease [2, 4]. In addition, the frequency of esophageal adenocarcinoma has increased by more than

✉ Rhonda Frances Souza
rhonda.souza@bswhealth.org

[1] Center for Esophageal Research, Baylor Scott and White Research Institute, 2 Hoblitzelle, Suite 250, 3500 Gaston Avenue, Dallas, TX 75246, USA

sevenfold in the past four decades in the United States [2]. Within Japan, estimates on the prevalence of short-segment Barrett's esophagus range from 1.2 to 59% and for long-segment from 0.2 to 1.4% [3]. Although Barrett's esophagus appears to be increasing in Japan, esophageal adenocarcinoma still accounts for <5% of esophageal cancer cases in this country [3]. In order to make advances in preventing Barrett's esophagus and the other serious esophageal complications of reflux esophagitis, a better understanding of the pathogenesis of reflux esophagitis and its contribution to the development of Barrett's esophagus is essential. This review will focus on current research concepts supported by recently published key studies on the development of reflux esophagitis and Barrett's esophagus.

Pathogenesis of reflux esophagitis: acid burn or cytokine sizzle

In 1935, gastroenterologist Asher Winkelstein reported in the Journal of the American Medical Association patients who had heartburn and other esophageal symptoms associated with endoscopic and histologic signs of inflammation in the distal esophagus. Winkelstein went on to propose that these patients had "peptic esophagitis…resulting from the irritant action on the mucosa of free hydrochloric acid and pepsin". For more than 80 years, this traditional concept that reflux esophagitis results from an acid-peptic "burn" has been a widely held belief that has for the most part gone unchallenged. In this model, reflux esophagitis is thought to start when refluxed acid and pepsin damage the proteins of the junctional complexes that bind cells together to make the epithelium impermeable to water, hydrogen ions, and other solutes [5]. However, when these junctional proteins are damaged, the epithelium becomes permeable, and allows acid to enter and attack the epithelial cells. This acid burn causes the death of surface epithelial cells, which triggers the infiltration of neutrophils and eosinophils and induces proliferation of esophageal basal cells, efforts that aid in repairing the injured epithelium (Fig. 1a) [6].

In 2008, our group began using a rat model in which reflux esophagitis was induced by creating a surgical esophagoduodenostomy. We noted that erosive esophagitis took weeks to develop after the surgical induction of reflux in our animal model. Esophageal injury due to an acid burn should develop rapidly, and we were puzzled by the long delay between the onset of reflux and the appearance of esophagitis. Using this animal model, we studied the histologic events of reflux esophagitis beginning at post-operative day 3 and 7 and then every week after out to post-operative week 8 with comparison to sham-operated control animals [7]. On post-operative day 3, we found esophageal inflammation most prominent in the submucosa, an esophageal mucosa that was intact, and that the inflammatory cell infiltrate in the submucosa was exclusively lymphocytes [7]. By post-operative week 1, the lymphocytic-predominant inflammation reached the lamina propria and by post-operative week 3, the epithelial layer was inflamed [7]. Using immunostaining for CD3, a T cell marker and CD20, a B cell marker, we found that the infiltrating lymphocytes were CD3+ and CD20−, demonstrating that they were T lymphocytes [7]. Basal cell proliferation (i.e., hyperplasia)

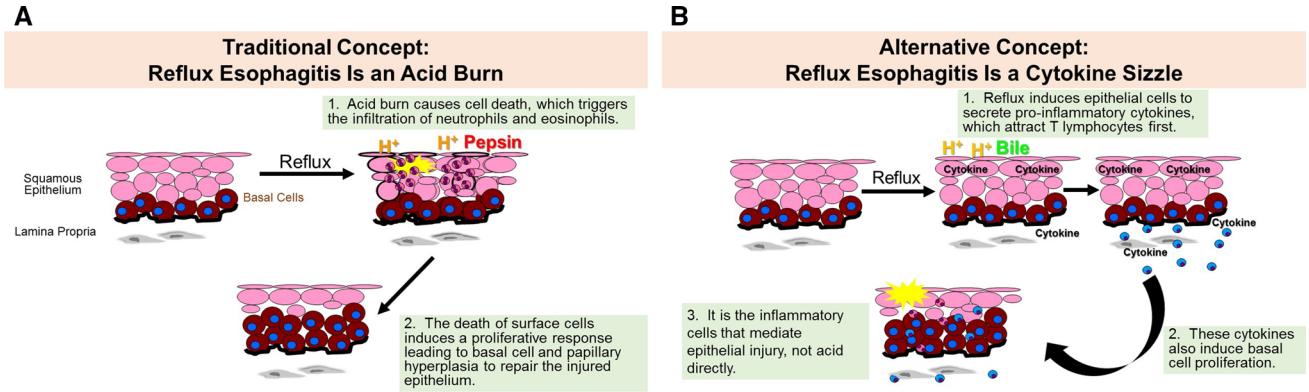

Fig. 1 Concepts on the pathogenesis of reflux esophagitis. **a** The traditional concept has been that reflux esophagitis results from a caustic (acid) burn. When esophageal squamous epithelium is exposed to reflux, acid and pepsin are thought to damage the junctions between the cells, making the epithelium permeable and allowing acid to seep into the epithelium and injure the epithelial cells. This acid burn causes cell death, which triggers the infiltration of neutrophils and eosinophils into the epithelium. The death of surface cells is also assumed to induce a proliferative response leading to basal cell and papillary hyperplasia to repair the injured epithelium. **b** The alternative concept that we propose is that reflux esophagitis develops as a cytokine-mediated inflammatory injury (i.e., cytokine sizzle). In this model, the reflux of acid and bile salts does not destroy epithelial cells directly, but rather induces them to secrete pro-inflammatory cytokines, which attract T lymphocytes first. These cytokines also induce basal cell proliferation. Ultimately, it is inflammatory cells that mediate the epithelial injury, not the direct acid burn

began at post-operative week 1 and peaked in degree by week 4, but it was not until week 4 that we began to see death of surface epithelial cells (i.e., erosions) [7]. In contrast to the acid burn model, we found in this animal model that inflammation did not start in the mucosa, and the first inflammatory cells were lymphocytes, not neutrophils or eosinophils. We also found that basal cell hyperplasia occurred while the surface epithelial cells were still intact, so this hyperplasia was not due to the death of surface cells [7].

Since our initial observation was the infiltration of a lymphocytic infiltrate, we postulated that the reflux of acid and bile induces esophageal epithelial cells to secrete pro-inflammatory cytokines. Using cultures of esophageal squamous cells derived from patients with GERD, we found that cells secreted interleukin (IL)-8 and IL-1β, potent pro-inflammatory cytokines, when they were exposed to acidic bile salts, and that secretion of IL-8 induced the migration of lymphocytes and neutrophils [7]. Expression of IL-8 by reflux-stimulated esophageal squamous cells was also observed in our animal model in vivo [7]. Based on these findings, we proposed an alternative concept for the pathogenesis of reflux esophagitis in which reflux esophagitis begins as a cytokine-mediated injury (i.e., cytokine sizzle) rather than a caustic chemical injury [7]. In this model, the reflux of acid and bile does not destroy epithelial cells directly, but rather induces them to secrete pro-inflammatory cytokines. These cytokines attract lymphocytes first, rather than neutrophils or eosinophils, and they induce the basal cell and papillary proliferation characteristic of GERD [5]. We postulate that, ultimately, it is inflammatory cells that mediate the epithelial injury through a cytokine sizzle, rather than the direct caustic effects of an acid burn (Fig. 1b).

Reflux esophagitis in humans: a likely result of the cytokine sizzle

Our alternative concept on the pathogenesis of reflux esophagitis was based on rat and cell lines studies, and it was not clear if this model is applicable to humans. Validation that acute reflux esophagitis in humans is cytokine-mediated (i.e., not primarily an acid burn) could have important implications for the prevention and treatment of GERD, but the logistics of conducting such a validation study are challenging. For example, GERD patients typically have years of symptoms before seeking medical attention [8], and physicians rarely, if ever, see patients with "acute" GERD. Thus, the early histologic changes of reflux esophagitis had not been evaluated prospectively in humans. It has been known for decades that severe, erosive reflux esophagitis healed by proton pump inhibitor (PPI) therapy will return in most cases within 6–12 months after PPIs are stopped, although the rapidity with which erosive esophagitis redevelops has not been clear [9, 10]. So, we induced acute reflux esophagitis by temporarily interrupting PPI therapy in patients with severe GERD [11]. Using an endoscopy database, we identified 12 patients with Los Angeles (LA) Grade C reflux esophagitis, and treated them with PPIs twice daily for at least 1 month [11]. While patients were taking their PPIs, we performed endoscopy using high-definition white light and confocal laser endomicroscopy (CLE) with biopsy of the distal esophagus, and we stopped the PPIs. At 1 and 2 weeks, we repeated the endoscopy and CLE with biopsy; at the end of week 2, we restarted the patients on their PPIs [11]. Within 2 weeks after stopping PPIs, all 12 patients had developed endoscopic reflux esophagitis with five patients developing LA Grade C in this short time period. At 1 and 2 weeks after stopping PPIs, significant increases were found in lymphocytes infiltrating the epithelium; neutrophils and eosinophils were few in number. Immunostaining for CD3 and CD20 demonstrated that the lymphocytes were almost exclusively CD3+ T cells, similar to our rat studies [11]. CLE imaging demonstrated significant increases in intercellular space width in the proximal and distal esophagus (i.e., dilation of intercellular space) and in capillary width within 2 weeks after stopping PPIs [11]. We also observed by CLE that the widened intercellular spaces contained increases in fluorescein, the intravenous contrast agent given to patients to enhance identification of cells and capillaries (Fig. 2). In the acid burn model for the pathogenesis of reflux esophagitis, dilation of intercellular spaces (DISs), a characteristic GERD feature, is thought to result from acid-induced damage of the proteins of the junctional complexes causing increases in epithelial permeability, which allow water to enter from the luminal surface and expand the intercellular spaces [12]. However, our observation that blood-borne fluorescein increases in the intercellular spaces suggests that perhaps DISs result from reflux-induced inflammation increasing vascular permeability, which allows for the leakage of fluid out of the blood vessels and into the intercellular spaces causing their expansion. Overall, our findings in GERD patients with acute reflux esophagitis induced by interrupting PPI therapy for 2 weeks are consistent with our earlier findings in our rat studies, suggesting that the pathogenesis of reflux esophagitis may be mediated by the cytokine sizzle rather than the acid burn (Fig. 1b).

Hypoxia-inducible factor (HIF)-2α: initiator of the cytokine sizzle

Inflamed tissues, such as reflux esophagitis, are often hypoxic, and hypoxia induces the expression of hypoxia-inducible factors (HIFs). HIFs are heterodimeric

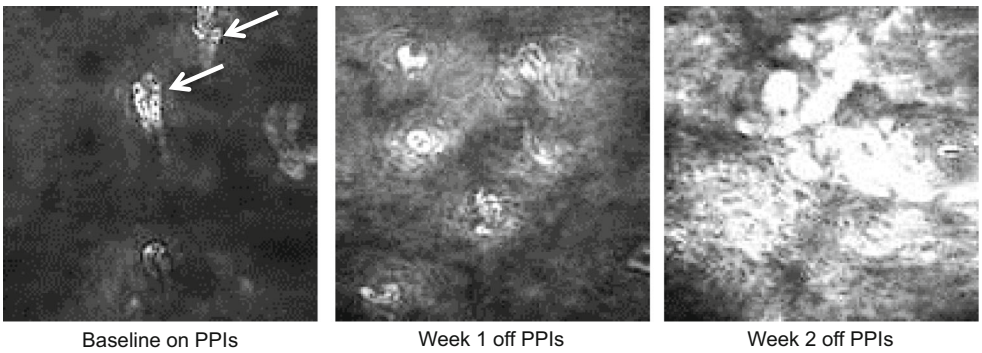

Fig. 2 Confocal laser endomicroscopy (CLE) images of acute reflux esophagitis. Representative images from the distal esophagus of an individual study subject at baseline on proton pump inhibitors (PPIs), week 1 and week 2 off of PPIs. *White arrows* indicate fluorescein within the intraepithelial capillaries at baseline on PPIs. By 1 and 2 weeks off PPIs, the fluorescein has leaked from the blood vessels into the intercellular spaces, enhancing the identification of the individual squamous cells. Images courtesy of Dr. Kerry B. Dunbar

transcription factors that have HIF-α subunits (either HIF-1α or HIF-2α), which are oxygen regulated and a HIF-1β subunit, which is constitutively expressed [13]. HIFs play a key role in enabling cells to respond to hypoxia stress and in mediating inflammatory processes [13–16]. Under normoxic conditions, HIFs are inactive because the HIF-a subunits are degraded by proteasomes. In the setting of hypoxia, however, proteosomal degradation is inhibited—the HIF-a subunits are stabilized, allowing for them to accumulate within the cell. The HIF-α subunit then binds to the HIF-1β subunit, they translocate to nucleus, and induce the transcription of target genes that contain hypoxia-responsive elements (HREs) [14, 17, 18].

In a mouse model of colonic inflammation, Shah et al. found that colonic inflammation developed in a pattern very similar to our rat model of esophagitis, with inflammation starting in the submucosa that subsequently progressed to the mucosal surface associated with an increase in proliferation and an increase in expression of pro-inflammatory cytokines [19]. Subsequent studies by this group demonstrated that it was HIF-2α, and not HIF-1α, that mediated the colonic inflammation in this mouse model [20]. In addition to hypoxia, HIF can be induced by the production of reactive oxygen species (ROS) and we have shown that human esophageal squamous cells in culture exposed to acid and bile salts increase the intracellular production of ROS [21]. Therefore, we reasoned that refluxed acid and bile salts may cause esophageal squamous epithelium to produce ROS, which activate HIF-2α to induce the expression of pro-inflammatory mediators. Indeed, using our cultured esophageal squamous cells, we found that exposure to acidic bile salts increased ROS production, increased HIF-2α expression and activity, and increased mRNA expression of pro-inflammatory molecules including T lymphocyte-attracting chemokines [22]. Moreover, we found that the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway was a major effector of the HIF-2α-mediated esophageal epithelial cell inflammatory response to acidic bile salts [22]. These findings in esophageal squamous cells in culture suggests that HIF-2α might play a role in inducing acute reflux esophagitis in our patients during the 2-week interruption of their PPI therapy [11].

Using esophageal biopsies from our patients, we found no change in squamous epithelial cell immunostaining for HIF-1α from baseline to 2 weeks after stopping PPIs. In contrast, we observed an increase in epithelial cell cytoplasmic and nuclear HIF-2α staining form baseline to 1 and 2 weeks after stopping PPIs [22]. Using an index called an H score [23, 24], we quantitated the HIF-1α and HIF-2α staining in all 12 patients. There was no significant change in squamous epithelial cells staining for HIF-1α from baseline to 2 weeks after stopping PPIs. In contrast, HIF-2α squamous epithelial cell staining significantly increased at 1 week after stopping PPIs and remained elevated at week 2 [22]. In esophageal biopsies from these same 12 patients, we also found increases in mRNA expression of the pro-inflammatory mediators including IL-8 and IL-1β. To determine if changes in HIF-2α protein expression were associated with changes in mRNA expression of the pro-inflammatory mediators, we computed eta^2 values, which are used for non-linear correlations [25]. Values of 0.14 or greater are generally interpreted as indicating a large association [25]. At week 1, we found large associations between the HIF-2α H score and mRNA expression levels of IL-1β and by week 2, large associations were found with IL-8 [22]. We next sought to determine whether HIF-2α enhanced NF-κB/p65 activity in human esophageal biopsies. Immunostaining for the active, phosphorylated form of p65 in esophageal biopsies demonstrated significant increases at week 1, which remained elevated at week 2 after stopping PPIs [22]. Non-linear correlations between H scores of HIF-2α and phosphorylated p65 demonstrated large associations between these proteins at 1 and 2 weeks

after stopping PPIs [22]. Furthermore, non-linear correlations demonstrated large associations between phosphorylated p65 and IL-8 and IL-1β at week 2 off PPI therapy [22]. Thus, our in vitro and in vivo findings have elucidated molecular mechanisms whereby the reflux of acid and bile salts causes esophagitis through cytokine-mediated mechanisms triggered by HIF-2α.

Barrett's esophagus: a serious complication of reflux esophagitis

Reflux esophagitis can lead to Barrett's esophagus, which develops through metaplasia. Metaplasia occurs when one adult tissue type replaces another, usually as a response to tissue damage and regeneration from chronic inflammation [26–28]. Metaplasia might represent a protective adaptation to chronic injury, but metaplasia also can predispose to malignancy for reasons that are not clear [29]. In the esophagus, chronic inflammation due to reflux esophagitis damages the squamous epithelium and allows for its replacement by an abnormal columnar epithelium (specialized intestinal metaplasia) comprising a mixture of gastric and intestinal cell phenotypes [2, 30]. This metaplastic epithelium is called Barrett's esophagus, and is a major risk factor for esophageal adenocarcinoma [31, 32].

The pathogenesis of this disorder remains poorly understood. A topic of intense research interest is the identity of the cell of origin for the specialized intestinal metaplasia of Barrett's esophagus. A number of potential sources have been proposed including the esophagus, the proximal stomach, or the bone marrow. However, intestinal cells are not normally found in the esophagus, the stomach, or the bone marrow. Thus, it would seem that Barrett's metaplasia results from a process called cellular reprogramming, in which the expression of key developmental transcription factors is altered in a way that changes a cell's phenotypic commitment [27]. It is generally accepted that GERD is the condition that induces the cellular reprogramming, but the identity of the progenitor cells whose reprogramming gives rise to Barrett's metaplasia remains unclear [27]. We will review some key studies addressing the mature esophageal squamous epithelial cell and the esophageal squamous epithelial progenitor cell as the potential origin of Barrett's metaplasia and the effects of noxious components found in gastroesophageal reflux on esophageal progenitor molecular reprogramming. However, these studies do not refute the alternative possibility that a columnar progenitor cell (in the gastric cardia or at the gastroesophageal junction) might also be a precursor of Barrett's metaplasia, nor are these possible origins mutually exclusive.

Origin of Barrett's esophagus: fully, differentiated esophageal squamous epithelial cells

Barrett's metaplasia may result from transdifferentiation, the process in which one fully differentiated cell type (i.e., squamous) changes directly into another (i.e., intestinal) [28]. Direct transdifferentiation is a molecular reprogramming event that does not require the cell to divide in order to change its phenotype (Fig. 3a) [28]. Explants of mouse embryonic columnar-lined esophagus grown in vitro have been shown to lose columnar cell markers and gain squamous cell markers, with a subset of cells simultaneously expressing both types of markers [33]. This switch in marker expression can occur without accompanying changes in cell death or proliferation, suggesting that one cell type can convert directly into another without any intermediary cell divisions [33, 34]. It is also possible that the cells with features of both cell types (transitional cells) represent de-differentiated cells that can reprogram into the new cell type through a series of intervening cell divisions (Fig. 3b) [28, 35].

Some studies on patients with Barrett's esophagus have suggested that a transdifferentiation process might underlie the pathogenesis of the esophageal metaplasia. Biopsy specimens taken at the squamo-columnar junction (SCJ) in Barrett's patients can show a "multilayered epithelium" with a basal layer of squamous cells covered by a superficial layer of columnar cells [36]. Immunocytochemical staining of this multilayered epithelium demonstrates that some cells display both squamous and columnar cell features [36], and scanning electron microscopy has demonstrated a "distinctive cell" at the squamocolumnar junction with ultrastructural characteristics of both squamous and columnar cells [37].

Origin of Barrett's esophagus: esophageal squamous epithelial progenitor cell

Despite this indirect evidence for transdifferentiation, it seems unlikely that the variety of gastric and intestinal cell types that comprise Barrett's metaplasia develop solely through the transdifferentiation of mature esophageal squamous cells [38]. It is more likely that a metaplasia, in which one tissue type converts into another, arises from undifferentiated progenitor cells that have the capacity to produce and maintain multiple cell types [28]. In the setting of GERD-induced tissue damage, immature progenitor cells are molecularly reprogrammed to express columnar rather than squamous developmental transcription factors, thereby differentiating into the multiple columnar cell types of Barrett's metaplasia. This molecular

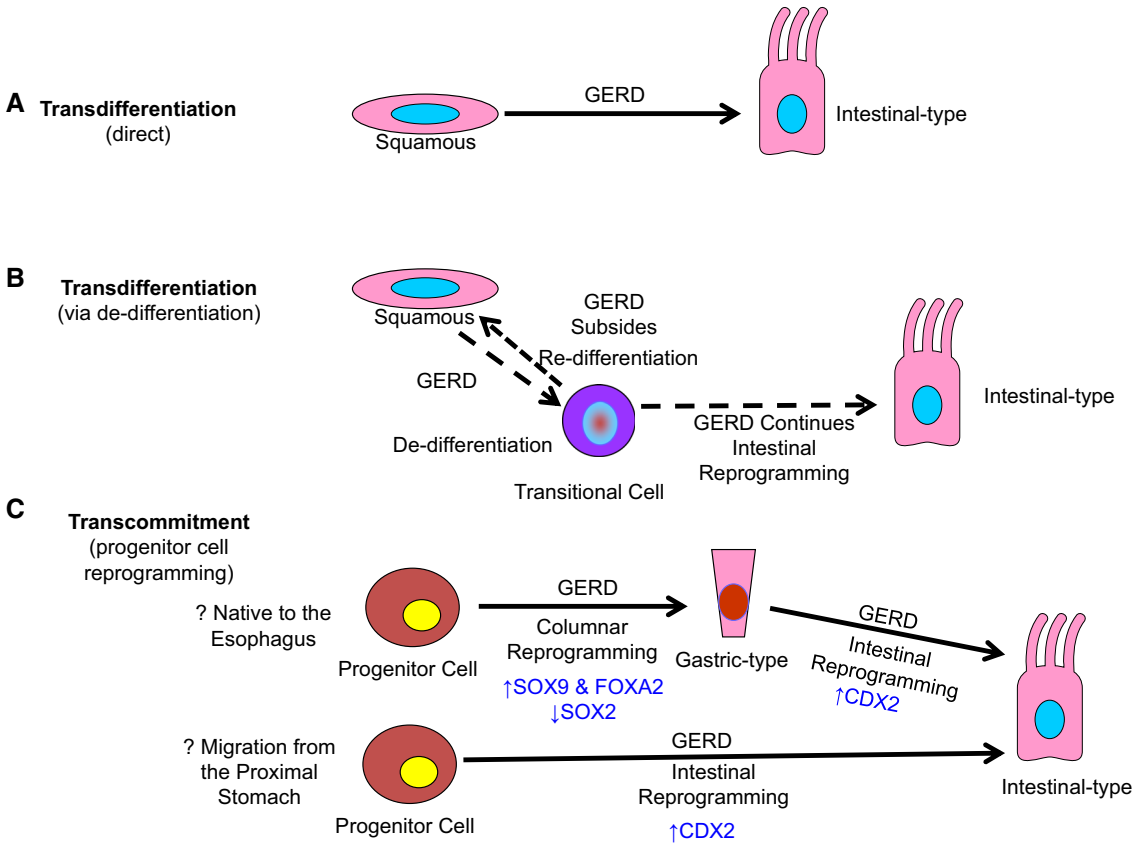

Fig. 3 Conceptual overview of GERD-induced cellular reprogramming in the pathogenesis of Barrett's metaplasia. Potential pathways for the origin of Barrett's metaplasia include (**a**) direct transdifferentiation is the process in which an individual, fully differentiated cell (i.e., squamous) change directly into another type of fully differentiated cell (i.e., intestinal-type cell) in the setting of GERD. **b** Transdifferentiation in the setting of GERD may result in de-differentiated cells with features of both squamous and intestinal cell types (transitional cells). If GERD subsides, this transitional cell can re-differentiate into a squamous cell. If GERD continues, this transitional cell can reprogram into the new intestinal-type cell through a series of intervening cell divisions. **c** Transcommitment is the process in which immature progenitor cells are reprogrammed in the setting of GERD to give rise to the gastric and intestinal cell types that comprise Barrett's metaplasia. Progenitor cells that are native to the esophagus undergo reprogramming to columnar gastric-type cells. Some of these gastric-type columnar cells undergo further reprogramming into intestinal-type cells. Progenitor cells may migrate from the proximal stomach into the esophagus, but some of these gastric progenitor cells would still have to undergo reprogramming into intestinal-type cells. Some of the transcription factors that have been implicated in these GERD-induced reprogramming processes are indicated in *blue*

reprogramming process has been called transcommitment, and the responsible progenitor cells might be native to the esophagus, or they might migrate into the esophagus from the proximal stomach when the squamous epithelium is damaged by GERD (Fig. 3c). In support of a molecular reprogramming process of squamous esophageal progenitor cells, immortalized esophageal squamous cell lines and tissues exposed to acid and bile salts in vitro or GERD in vivo increase their expression of the columnar transcription factors SOX9 and forkhead box protein A2 (FOXA2), which are targets of the Hedgehog pathway, and of the intestinal transcription factor caudal-related homeobox transcription factor 2 (Cdx2), a target of the (NF-κB) pathway (Fig. 3c) [39–43]. In addition, esophageal squamous cells exposed to nitric oxide, another noxious component of reflux, in vitro or in vivo, decrease their expression of sex-determining region Y-box 2 (SOX2), a transcription factor that promotes stratified squamous epithelia development, through inhibition of protein kinase B (PKB or Akt) pathway signaling (Fig. 3c) [44, 45].

GERD-induced columnar transcription factors SOX9 and FOXA2: targets of the hedgehog pathway

SOX9 and FOXA2 are transcription factors that characterize columnar cells and both are targets of the Hedgehog (Hh) pathway. Hh ligands bind to their transmembrane receptor called patched (PTCH) to activate pathway signaling. In the absence of ligand binding, PTCH inhibits smoothened (Smo), a protein that transduces the signal

downstream. Following the binding of Hh to PTCH, Smo is released from PTCH inhibition and activates Gli transcription factors to regulate downstream target genes [40]. Wang et al. demonstrated that esophageal squamous cell lines and squamous tissues exposed to acid and bile salts in vitro or to gastroesophageal reflux in vivo exhibit Hedgehog pathway signaling. Following exposure to acid and bile salts, esophageal squamous epithelial cells secrete the hedgehog ligand, sonic hedgehog. The secretion by the epithelial cells of this ligand binds the PTCH receptor located on stromal fibroblasts leading to the secretion of bone morphogenic protein 4 (BMP4). BMP4 then binds to its receptors, the BMP type I receptors located on the esophageal squamous cells and this epithelial–mesenchymal Hh signaling causes the squamous cells to produce the transcription factor SOX-9 [39]. Furthermore, esophageal squamous cells expressing plasmids containing sonic hedgehog, Gli1, or a constitutively active BMP receptor type IA induced the columnar cell transcription factor FOXA2 [40]. Expression of SOX-9 and FOXA2 induces genes that influence columnar and goblet cell differentiation such as cytokeratin 8 and mucin 2 (MUC2) [39, 40]. Thus, reflux of acid and bile salts could initiate reprogramming of progenitors in the esophagus by activation of Hedgehog signaling and upregulation of SOX9 and FOXA2.

The GERD-induced intestinal transcription factor Cdx2: target of the NF-κB pathway

Cdx2 is a key developmental transcription factor that directs formation of intestinal epithelium and is a target of the NF-κB pathway [46, 47]. In fact, two putative NF-κB binding sites have been identified in the Cdx2 promoter [48]. Uninflamed esophageal squamous epithelium does not express active NF-κB, but expression of the activated form of this transcription factor has been found in the esophageal epithelium as reflux-induced inflammation ensues [22, 49]. In previous studies, we demonstrated that acid and bile salt exposure induces NF-κB signaling in esophageal squamous cells in culture [22, 43, 50]. In the pancreas, furthermore, NF-κB signaling has been shown to play a key role in the molecular reprogramming process that underlies pancreatic acinar-to-ductal metaplasia [51].

CdX2 expression is frequently found in biopsy specimens of Barrett's metaplasia, which is not surprising since intestinal-type columnar cells are characteristic of this esophageal metaplasia [52–55]. Like NF-κB, CDX2 expression has been found in biopsy specimens of esophageal squamous epithelium inflamed by GERD, but not in uninflamed esophageal epithelium [55]. In animal models of reflux esophagitis, the reflux-damaged esophageal epithelium increases CDX2 expression before the development of a Barrett's-like metaplasia [44, 56, 57]. In cultured esophageal squamous cells from rats and some human subjects, acid and bile salt exposures have also been shown to increase activity of the Cdx2 promoter and increase Cdx2 mRNA expression [58–61]. Tamagawa et al. demonstrated that bile salts increase CDX2 expression, which in turn decreases HES1 and increases atonal homolog 1 (ATOH1) expression, targets that reflect decreases in Notch pathway signaling [62, 63]. Moreover, the combination of increased CDX2 and decreased Notch signaling led to increases in MUC2 and delta-like 1 (Dll1), genes that further promote goblet cell differentiation [63]. These studies suggest that the reflux of acid and bile salts could initiate reprogramming of progenitors in the esophagus by activation of NF-κB signaling and upregulation of Cdx2.

GERD-suppressed stratified squamous epithelial transcription factor SOX2: a target of the Akt pathway

The majority of studies on the molecular events underlying the intestinal metaplasia of Barrett's esophagus have focused primarily on the upregulation of genes involved in columnar and intestinal differentiation such as Sox9 and Cdx2 [39, 43]. However, it seems equally plausible that the molecular reprogramming of squamous-to-columnar metaplasia also involves the downregulation of genes that regulate squamous differentiation such as Sox2 and the isoforms of tumor protein p63 (p63) [64, 65]. Furthermore, noxious components of gastroesophageal reflux, other than acid and bile salts, like nitric oxide (NO), may play a role in the reprogramming process.

Iijima et al. found that high concentrations of NO can be generated in the esophageal lumen during episodes of gastroesophageal reflux [66]. Dietary nitrate is commonly found in green, leafy vegetables. When these foods are ingested, dietary nitrate is absorbed and secreted into the saliva. Oral bacteria then reduce the nitrate to nitrite, which is then swallowed. When nitrite comes into contact with refluxed gastric acid, NO is rapidly generated. This NO can react with oxygen to form highly toxic reactive nitrogen species that result in damage to the tissue [67]. In GERD patients with and without Barrett's esophagus, NO generated from dietary nitrate has been shown to reach genotoxic concentrations at the gastroesophageal junction [68]. Moreover, Endo et al. found that dietary supplementation with nitrates accelerated the development of metaplasia in a rat model of reflux esophagitis [69]. However, very little had been known regarding the mechanisms whereby exposure of the esophagus to NO, generated from dietary

nitrate, might facilitate the development of Barrett's metaplasia.

Using esophageal squamous cells in culture, Asanuma et al. found that exposure to the small molecule NO donor, NOC9, profoundly reduced SOX2 mRNA expression compared to cells exposed to acid and bile salts [45]. NOC9 exposure caused S-nitrosylation of Akt, which blocked its phosphorylation, and interfered with its downstream signaling, leading to reductions in SOX2 mRNA and protein [45]. Moreover, the generation of NO by NOC9 decreased the expression of the TA and ΔNP isoforms of p63, another transcription factor that promotes stratified squamous epithelia, and increased the expression of CDX2 [65, 70]. Using tissue specimens from rats with surgically induced reflux esophagitis fed postoperative diets with and without NO supplementation, the investigators found diminished staining for SOX2 in the squamous-lined distal esophagus of rats fed an NO-supplemented diet compared to rats feed a normal diet [45]. These findings suggest that the generation of NO by gastroesophageal reflux could initiate reprogramming of progenitors in the esophagus by inhibiting Akt signaling causing reduction in SOX2, by decreasing the TA and ΔNP isoforms of p63, and by upregulating CDX2, events that might lead to the development of the intestinal metaplasia of Barrett's esophagus.

Conclusions

Data from a rat model and humans suggest that reflux esophagitis develops as a cytokine-mediated inflammatory injury (i.e., sizzle), not as a caustic chemical injury (i.e., acid burn), as has traditionally been assumed. Molecular mechanisms elucidated in esophageal squamous cell lines demonstrate that acid and bile salts, the major components of gastroesophageal reflux, induce HIF-2α, which enhances NF-κB transcriptional activity resulting in the production of pro-inflammatory molecules including chemokines that attract T lymphocytes. Esophageal biopsies of patients with acute reflux esophagitis at 1 and 2 weeks after stopping PPIs demonstrate large associations between HIF-2α production, NF-κB activation, and pro-inflammatory mediator expression in support of such a mechanism. The past few years have seen an explosion of research into the origin of Barrett's esophagus, and controversy currently exits as to whether GERD-induced molecular reprogramming of progenitors that are native to the esophagus is involved. Investigations into this issue have uncovered the role of signaling pathways like Hedgehog, NF-κB, and Akt and transcription factors like SOX9, FOXA2, CDX2, and SOX2 that conceivably could induce squamous-to-columnar molecular reprogramming. Thus, new insights into understanding the pathogenesis of reflux esophagitis and reflux-related reprogramming of native esophageal progenitors have highlighted potential molecular pathways and molecules for future targeted therapies to prevent the development of Barrett's esophagus.

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Compliance with ethical standards

Conflict of interest The author declares that she has no conflicts of interest.

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