New Concepts in Gastroesophageal Reflux Disease and Barrett's Esophagus

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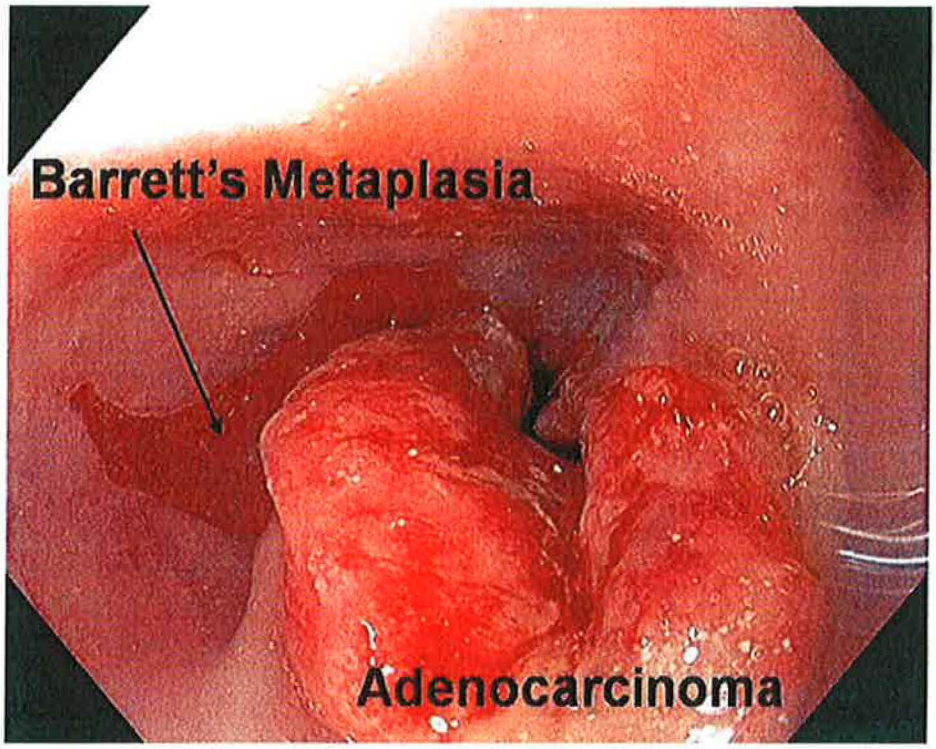

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My major interests are in the molecular mechanisms underlying the development and neoplastic progression of Barrett's esophagus. In our laboratory, we utilize biopsy tissues, cell culture systems, and animal models to delineate the signal transduction pathways activated by acid, bile, and the combination of both in esophageal squamous and metaplastic Barrett's epithelia. In addition, the downstream effects on proliferation and apoptosis of these activated signal transduction pathways are investigated in an effort to identify potential targets at which to direct chemopreventive and chemotherapeutic agents. We have also transformed Barrett's epithelial cells by the introduction of well-defined genetic alterations and we are using these unique cell lines to study cancer-related inflammation and Barrett's carcinogenesis.

Introduction

The major risk factors for esophageal adenocarcinoma are gastroesophageal reflux disease (GERD) and Barrett esophagus, a squamous-to-columnar cell metaplasia that predisposes to malignancy. Esophageal adenocarcinoma is one of the most deadly gastrointestinal cancers. Despite ongoing therapeutic advances, the mortality rate for this tumor remains above 90% (2). Especially concerning is the 6-fold increase in the incidence of esophageal adenocarcinoma over the past three decades (3). Although GERD and Barrett's esophagus are the major risk factors for esophageal adenocarcinoma, the precise mechanisms that underlie the development of Barrett's metaplasia and its progression to malignancy remain poorly understood.

For patients with Barrett's esophagus, endoscopic surveillance for dysplasia is the primary strategy recommended to decrease morbidity and mortality from esophageal adenocarcinoma (4). This strategy has not been effective, however, as evidenced by the continued rise in the frequency of esophageal adenocarcinoma, and by a recent study showing that more than 95% of patients with this tumor have no prior diagnosis of Barrett's esophagus and, thus, are not enrolled in surveillance programs (5). Effective strategies for the management and prevention of colorectal cancer have emerged from basic investigations that have elucidated the genetic events underlying colonic carcinogenesis (6). Unfortunately, the molecular events underlying the transformation of esophageal squamous cells into metaplastic Barrett's columnar cells, and the transformation from benign Barrett's metaplasia into adenocarcinoma remain unclear (7). It is important to understand these processes at the molecular level in order to identify biomarkers which may enable the selection of a subgroup of patients who would benefit from therapies to prevent the development of Barrett's esophagus and thus esophageal adenocarcinoma.

Investigators have used a number of endoscopic techniques to ablate Barrett's metaplasia in an attempt to eliminate the cancer risk (8). Recently, there has been intense interest in the use of radiofrequency energy ablation of Barrett's metaplasia using the HALO System (BARRX Medical, Inc.) (9). After ablation, patients are given potent antireflux therapy (usually PPIs) so that the injured mucosa heals with the growth of neo-squamous epithelium rather than with the regeneration of more Barrett's epithelium. Such ablation can be cost-effective only if Barrett's metaplasia does not re-develop after the procedure. Thus elucidation of molecular pathways involved in the pathogenesis of Barrett's esophagus might identify molecular targets for therapies designed to prevent the development of metaplasia. Such therapies may prevent Barrett's esophagus from re-developing in patients who have had endoscopic ablation of Barrett's epithelium.

This protocol will review the current concepts in the pathogenesis and treatment of gastroesophageal reflux disease, surveillance and screening for Barrett's esophagus, and recent recommendations regarding endoscopic ablation of Barrett's epithelium. Some emerging concepts regarding the molecular mechanisms whereby GERD causes reflux esophagitis and Barrett's esophagus will also be reviewed.

Gastroesophageal Reflux Disease: Pathogenesis and Treatment

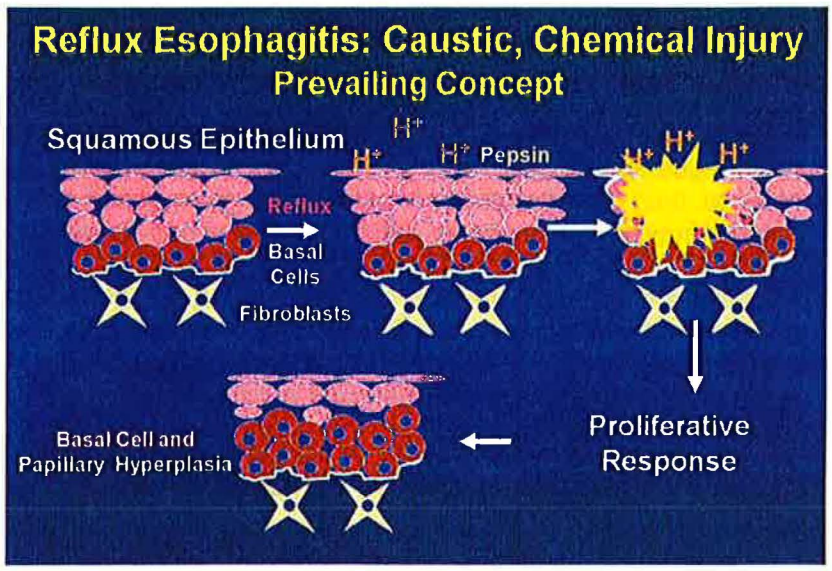
Current Concepts on Pathogenesis of Gastroesophageal Reflux Disease

GERD has been defined as "a condition that develops when the reflux of stomach contents causes troublesome symptoms and/or complications" (1). Here the key word is "troublesome" in referring to symptoms as this distinguishes GERD from just simple, occasional bouts of

heartburn (1). Among the primary causes of "troublesome" symptoms is the development of reflux esophagitis with ulcerations in the distal esophagus. In the US, GERD is extremely prevalent with approximately 20% of adults having heartburn more than once per week (10). Each year, more than 9 billion dollars are spent on medical evaluation and treatment for GERD so clearly this disease has a substantial impact on US healthcare (11). As I mentioned, one of the primary causes for troublesome GERD symptoms is reflux esophagitis.

For almost 100 years, the prevailing concept has been that reflux esophagitis results from a caustic, chemical injury that starts at the luminal surface. With reflux, acid and pepsin, components of gastric juice, are thought to damage the tight junctions between cells causing the intercellular spaces to dilate. This allows acid to enter the epithelium. As reflux continues, since this is thought to be a chemical burn, neutrophils are assumed to infiltrate the epithelium in response to acid-induced destruction of the surface cells. As the injury progresses through the epithelium and the surface cells continue to die, this is thought to trigger a proliferative response leading to basal cell and papillary hyperplasia to replace the refluxed-damaged squamous cells (Figure 1).

Figure 1. Prevailing Concept of Pathogenesis of Reflux Esophagitis

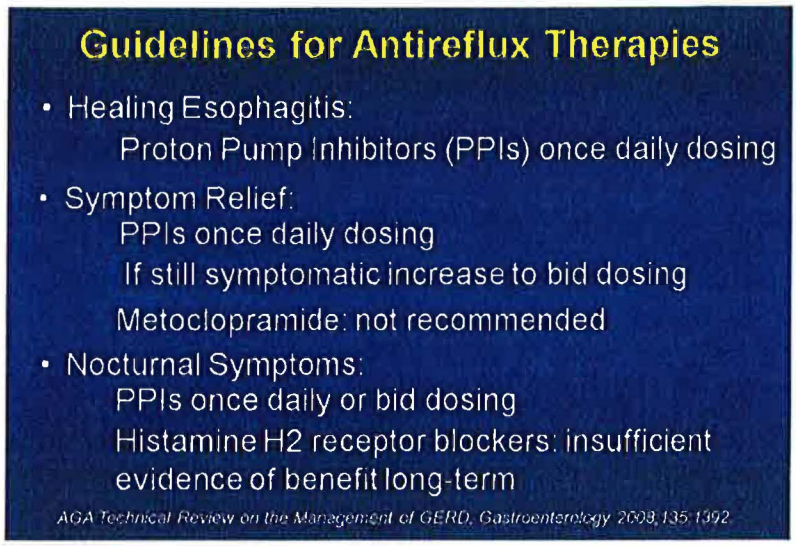


Current Treatment of Gastroesophageal Reflux Disease

Based on this model of reflux esophagitis, the main stay of therapy is to decrease acid secretion using agents like proton pump inhibitors (PPIs). The AGA Technical Review on GERD Management recommends that once daily dosing of PPIs be used both for healing esophagitis and for providing symptomatic relief (Figure 2)(1). If symptoms persist in patients on once daily dosing, then the dose should be increased to bid (1). Another drug that had traditionally been used to decrease acid exposure was metaclopramide or Reglan. Virtually, no data support the use of metoclopramide and in fact the available data recommend against its use because of its side effects (1). Finally, to control acid secretion at night for patients with nocturnal symptoms, once daily PPI is recommended with increasing to bid if symptoms persist. Current data are insufficient regarding the long term utility of adding a nocturnal dose of an H2 blocker to bid PPI therapy (1). As you can see, these therapies are directed solely at reducing gastric acid production to prevent caustic injury, but what if acid-induced caustic injury is not primary culprit

in the pathogenesis of reflux esophagitis?

Figure 2. Guidelines for Antireflux Therapies from the AGA Technical Review on the Management of GERD (1).



Emerging Concepts in Gastroesophageal Reflux Disease: Pathogenesis and Treatment

Emerging Concepts in the Pathogenesis of Gastroesophageal Reflux Disease

If caustic, chemical injury underlies the development of reflux esophagitis then damage to the esophageal mucosa at the luminal surface should develop rapidly and then progress to the deeper layers of the epithelium. However using a rat model of reflux esophagitis, our group was puzzled by the fact that erosive esophagitis often didn't appear for weeks after the operation that immediately resulted in reflux. So if reflux esophagitis is indeed a caustic chemical injury, then why the long delay between the onset of reflux and the appearance of esophagitis? To further investigate this observation, we used a rat model in which gastroesophageal reflux was surgically induced by performing an esophago-duodenostomy to study the early histologic events in the development of reflux esophagitis (12).

In sham-operated animals, no inflammation was observed in the epithelium, the lamina propria, or the submucosal layers of the distal esophagus (12). Three days after the surgical induction of reflux, the surface epithelial cells remained undamaged and the only apparent inflammation was the appearance of lymphocytes in the submucosa (12). By one week post-operatively, basal cell hyperplasia was observed even though the surface cells still remained intact and there were no surface erosions. Inflammatory cells, predominately lymphocytes, were present in the lamina propria as well as in the submucosa, but now neutrophils were found as well (12). Finally by 8 weeks after esophago-duodenostomy all the features of reflux esophagitis were observed. There was prominent inflammation of the submucosa and lamina propria that had progressed up to the epithelial layer, hyperplasia of the basal cell zone, papillary hyperplasia, and finally surface erosions (12).

Our observations in the animal model therefore do not support the concept that reflux esophagitis develops as a caustic, chemical injury that starts at the epithelial surface and moves to the deeper layers. Instead, these observations suggest that reflux esophagitis develops as an immune mediated injury. In our animal model, we found that reflux esophagitis starts as a lymphocytic infiltrate in the submucosa that progresses toward the mucosal surface. This is followed by the infiltration of neutrophils. There is also basal cell and papillary hyperplasia that clearly precedes surface erosion. This is exactly the opposite sequence predicted by the

conventional model of reflux eosphagitis as a caustic injury. Since the initial event appears to be immune cell infiltration, our findings suggested that perhaps gastroesophageal reflux causes esophageal squamous cells to produce cytokines.

Gastroesophageal reflux causes the production of cytokines by esophageal squamous cells.

To explore whether reflux might cause esophageal squamous cells to release cytokines, we used telomerase-immortalized normal esophageal squamous cell lines developed in our lab (12). We exposed the squamous cells to a combination of acid and conjugated bile acids for 10 minutes, 3 times a day for 5 days to simulate physiologic reflux (13). We then determined the amounts of various cytokines reported to be increased in biopsy tissues from patients with reflux esophagitis, in the conditioned media of the acid and bile acid treated esophageal squamous cells. Among the cytokines studied, we found that levels of IL-8 were significantly increased in the conditioned media after 2 days and continued to increase through day 5 of acid and bile acid exposure (12). To determine whether IL-8 was also produced by esophageal squamous epithelial cells in vivo, we performed IHC for IL-8 expression. Similar to our squamous cells in culture, we found expression of IL-8 by esophageal squamous cells in vivo. IL-8 was detected in the cytoplasm of the cells and in the intercellular spaces between the esophageal squamous cells at the luminal surface (12). We also found that the cytokines released by the acid and bile acid exposed esophageal squamous cells significantly increased T cell migration rates. However, the addition of an IL-8 blocking antibody to the conditioned media did not result in a significant decrease in T cell migration suggesting that IL-8 secretion by esophageal squamous cells is not the sole cytokine responsible for T cell migration (12). We performed similar experiments to determine the effect of IL-8 on neutrophil migration. We found that the conditioned media from esophageal squamous cells exposed to acid and bile acids significantly increased neutrophil migration rates. The addition of an IL-8 blocking antibody prevented the increase in neutrophil migration suggesting that IL-8 secretion by esophageal squamous cells induces the migration of neutrophils (12).

Alternative Concept for the Pathogenesis of Reflux Esophagitis

Our findings support an alternative concept in which reflux esophagitis begins as an immune-mediated injury. In the rat model, gastroesophageal reflux causes the esophageal squamous epithelial cells to produce and secrete chemokines. These chemokines induce the migration of lymphocytes initially to the submucosa with subsequent progression to the mucosal surface. This is followed by the infiltration of neutrophils. There is also basal cell and papillary hyperplasia that precedes surface erosion (Figure 3).

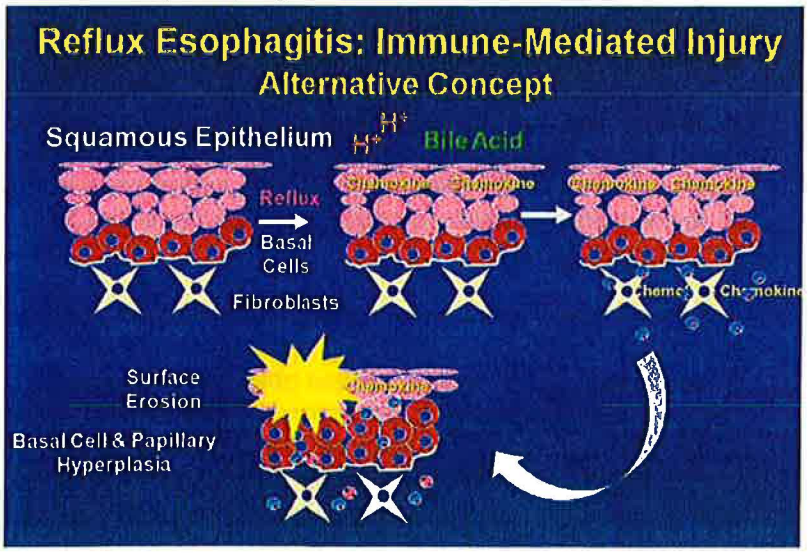


Figure 3. Alternative Concept for the Pathogenesis of Reflux Esophagitis.

This sequence is exactly the opposite of what would be expected from the traditional concept that reflux esophagitis results from a caustic, chemical injury. We don't know whether this is applicable to humans, but it might be. If so, then we may consider modifying our management strategy for patients with GERD which currently is directly solely at reducing acid secretion.

Emerging Concepts in the Treatment of Gastroesophageal Reflux Disease

Our studies in the rat model of reflux esophagitis found that the combination of acid and bile acids induces esophageal squamous cells to produce IL-8 which in turn causes the migration of neutrophils and lymphocytes. In esophageal biopsy specimens from patients with erosive esophagitis as well as in patients with non-erosive reflux disease (NERD), IL-8 and a number of other inflammatory cytokines have been found to be expressed by the epithelial cells (14-17). Moreover, high levels of IL-8 expression correlated with basal cell hyperplasia and the presence of intraepithelial neutrophils in biopsy tissues from patients with NERD (17). Thus, it is conceivable that an alternative, novel approach to GERD therapy in the future may be the targeting of IL-8 or the molecular pathways upstream of IL-8, activated by the components of gastric reflux, that lead to its production and secretion (18).

Barrett's Esophagus: A Complication of Gastroesophageal Reflux Disease

One question that commonly comes up when managing patients with GERD is "when should diagnostic tests be used in the evaluation of GERD symptoms." The AGA Technical Review on the Management of GERD recommends that "in the simplest case, when symptoms are typical and the patient responds to therapy intended to address those symptoms, no diagnostic tests are requisite"(1). The AGA Technical Review goes on to suggest that "diagnostic testing be invoked in 3 broad scenarios: (1) to avert misdiagnosis (2) to identify complications of reflux disease, and (3) in the evaluation of empirical treatment failures" (1). So, what are some of the complications of reflux disease that should not be missed? Gastroesophageal reflux can cause ulceration of the esophageal mucosa. Esophageal ulceration may stimulate a fibrotic reaction during healing resulting in stricture formation. In some patients, this reflux damaged squamous lining of the esophagus is replaced by an abnormal epithelium termed Barrett's esophagus, which predisposes to esophageal adenocarcinoma.

Current *Concepts in Endoscopic Surveillence for Neoplasia in Barrett's Esophagus*

The American College of Gastroenterology (ACG) recommends performing regular endoscopic surveillence of patients with Barrett's esophagus to detect early, curable neoplasia (4). Active inflammation can result in cellular atypia which can be mistakenly identified as dysplasia. Therefore, patients with GERD should be treated prior to surveillance endoscopy with PPIs to eliminate esophageal acid exposure (4). Since the distribution of dysplasia is often focal within the Barrett's mucosa, a technique of systematic, four-quadrant biopsies taken every 1-2 cm throughout the entire length of the metaplastic mucosa is recommended (4). Biopsies should be obtained from any areas of mucosal abnormality such as nodules or ulcerations. The grade of dysplasia within the metaplastic mucosa will determine the interval for subsequent surveillance endoscopies (4). However, despite surveillence programs, the incidence of esophageal adenocarcinoma remains the most rapidly rising, even when compared to melanoma, prostate, breast, lung, and colorectal cancer (3). So clearly our approach to these patients is not ideal. A meta-analysis by Dulai et al. provides insight into why this may be the case. In this study, the investigators looked at a number of individual studies where patients

were having an esophagectomy for esophageal adenocaricnoma and determined in each study the percentage of patients known to have Barrett's esophagus before they presented with symptoms of esophageal cancer (5). Overall, only 5% of patients were known to have Barrett's esophagus before being diagnosed with esophageal adenocarcinoma (5). The vast majority of cancer patients was not known to have Barrett's esophagus and thus were not enrolled in surveillance protocols. So clearly our screening programs to identify those patients with Barrett's esophagus are not effective. Such data have lead to an emphasis on now finding screening strategies to detect patients with Barrett's esophagus. However controversy exists on exactly which individuals should be screened for Barrett's esophagus.

Current Concepts in Endoscopic Screening for Barrett's Esophagus

The 2002 ACG practice guidelines recommend that patients with chronic GERD symptoms, who are the ones most likely to have Barrett's esophagus, should undergo upper endoscopy for screening purposes (19). However, in 2008 the ACG revised this recommendation stating that "the use of screening in selective populations at higher risk remains to be established and therefore should be individualized" (4). Similarly the recent AGA Technical Review on the Management of Barrett's Esophagus states that "inadequate evidence exists to endorse screening for Barrett's esophagus based solely on the presence of GERD symptoms and, decisions on when to recommend endoscopic screening should continue to be individualized" (20). These more recent recommendations shift the focus away from screening only patients with chronic GERD symptoms, a practice whose utility had come into question over the past few years. For example, Rex et al. determined the presence of Barrett's esophagus by upper endoscopy in a cohort of 961 patients who were scheduled for an elective colonoscopy (21). These patients had no prior history of having an upper endoscopy (21). A history of GERD symptoms was obtained and biopsies were taken if columnar mucosa extended $\geq$ 5mm into the distal esophagus (21). Barrett's esophagus was found in 6.8% of patients, including 1.2% with long segment ($\geq$3 cm) Barrett's esophagus and the majority (5.5%) with short segment ($<$3 cm) Barrett's esophagus (21). Among the 556 patients who had no history of heartburn symptoms, Barrett's esophagus was found in 5.6 %, with only .36% having long segment Barrett's (21). In those patients who had a history of heartburn, Barrett's esophagus was identified in 8.3% including 2.6% with long segment Barrett's (21). However, the frequency of short segment Barrett's esophagus identified in those without heartburn (5.2%) was almost the same as those with heartburn (5.7%)(21). These patients would be missed by the earlier guidelines suggesting that only patients with GERD undergo screening. So clearly there needs to be a better way to identify which individuals have Barrett's esophagus.

Emerging Concepts in Barrett's Esophagus: Pathogenesis and Treatment

Emerging Concepts in the Pathogenesis of Barrett's Esophagus

In order to identify those with Barrett's esophagus, we need to understand how this abnormal, metaplastic epithelium develops. Barrett's esophagus develops through metaplasia, the process in which one adult cell type replaces another. In the esophagus, the normal stratified squamous epithelium is replaced by specialized intestinal metaplasia characteristic of Barrett's esophagus. One way in which this metaplastic process could happen is through transdifferentiation, the process of changing the differentiation pattern of already differentiated cells. In general, metaplasias arise between tissue types present in the same organ during embryological development (22). In the esophagus, the cells initially lining the mucosa are of a columnar phenotype due to the expression of certain genes induced by high levels of morphogenic stimuli present early on during *in utero* development (22). As development proceeds and levels of

these morphogenic stimuli decline, there are changes in gene expression such that the columnar lining of the esophagus is replaced by a stratified squamous epithelium (23; 24). Therefore, by altering the pattern of gene expression, it is possible for the esophagus to change between a squamous and a columnar type of epithelial lining.

Bone morphogenic protein-4 (BMP-4) is expressed by stromal tissue in the embryonic columnar-lined esophagus, but not by that in the adult squamous-lined esophagus (25; 26). In a rat model of GERD and Barrett's esophagus, BMP-4 expression has been localized to the stromal tissue underlying the inflammed esophageal squamous epithelium and metaplastic Barrett's epithelium (26). No expression of BMP4 was found in the stroma underlying normal, non-inflammed squamous epithelium (26). Biopsy tissues from patients with GERD and Barrett's esophagus confirmed the animal data on the expression and localization of BMP-4 in these conditions suggesting that gastroesophageal reflux can induce stromal BMP-4 expression (26). Exposure of esophageal squamous cells in culture to BMP-4 causes the epithelial cells to express a number of columnar-like genes suggesting that stromal expression of BMP-4 stimulates the epithelial cells to change from a squamous to a columnar phenotype (26). One downstream target of BMP-4 signaling is the Caudal homeobox gene CDX2.

Homeobox (HOX) genes are key developmental genes that encode transcription factor proteins. CDX2 is a homeobox gene involved in determining an intestinal-like phenotype. CDX2 is not normally expressed in gastric columnar epithelial cells. When mice are genetically engineered so that CDX2 expression is specifically targeted to the gastric cells, the stomach develops a metaplastic, intestinal-type of epithelium (27). CDX2 expression has been detected in biopsy tissues of Barrett's metaplasia, but not in normal esophageal squamous epithelium (28). CDX2 expression has also been found in erosive esophagitis, but not in uninflammed, normal squamous mucosa (29). In a rat model of intestinal metaplasia, the squamous cells express cdx2 prior to the development of Barrett's esophagus (30). Finally, in 6 of 19 patients with Barrett's esophagus, their squamous epithelial cells were found to express CDX2 (31). Taken together, these findings suggest that CDX2 expression in esophageal squamous mucosa precedes the development of Barrett's esophagus.

Barrett's esophagus is a complication of chronic GERD, as discussed earlier, and CDX2 expression appears to underlie the formation of Barrett's metaplasia. There are at least two ways in which GERD might induce the expression of CDX2 in the esophagus. The components of refluxed gastric juice (e.g. acid and bile acids) or the resulting esophageal inflammation (reflux esophagitis) may 1) induce transcription factors like CDX2 directly in the esophageal epithelial cells or 2) activate developmental signaling pathways like BMP-4 that in turn induce transcription factors that determine an intestinal-like phenotype (32). Our group has recently explored the direct effects of acid and bile acids on CDX-2 mRNA and protein expression in esophageal squamous cell lines established from GERD patients with and without Barrett's esophagus (33). In the two esophageal squamous cell lines from Barrett's patients, there was an induction of CDX-2 mRNA and protein expression by acid alone, bile acids alone, and the combination of both. In contrast in the two squamous cell lines from GERD patients without Barrett's, there was no induction of CDX-2 mRNA or protein by any of the exposures. To determine if the CDX2 protein was functionally active in the esophageal squamous cells from patients with Barrett's esophagus, we determined mRNA expression levels of CK20, a CDX2 target gene. We found there was an induction of CK20 mRNA by acid alone, bile acids alone, and the combination of both suggesting that in fact the CDX2 was functionally active in these esophageal squamous cell lines. To confirm that the molecular events identified *in vitro* recapitulate human tissue *in vivo*, we studied CDX2 mRNA expression in esophageal squamous mucosal biopsy specimens taken during endoscopic examinations in GERD patients. We found

CDX2 mRNA expression in 7 of 10 squamous biopsy specimens from patients with Barrett's esophagus, whereas only 1 of 10 such specimens from patients who had GERD without Barrett's esophagus expressed CDX2 mRNA supporting our *in vitro* findings. Thus our data demonstrate that acid and bile acids can directly induce expression of CDX2 in esophageal squamous cells. Moreover, esophageal squamous cells from GERD patients with and without Barrett's esophagus differ in acid- and bile acid-induced expression of CDX2 suggesting that such differences might underlie the development of Barrett's metaplasia in some GERD patients but not in others.

The components of refluxed gastric juice (e.g. acid and bile acids) or the resulting esophageal inflammation (reflux esophagitis) may also activate developmental signaling pathways like BMP-4 that in turn induce transcription factors (e.g. CDX2) that determine an intestinal-like phenotype (32). Among the developmental signaling pathways implicated in the formation of Barrett's metaplasia is the Hedgehog pathway. Sonic hedgehog (Shh), the most abundant Hedgehog ligand, is expressed by the embryonic columnar-lined esophagus prior to changing into a stratified squamous lined adult esophagus (34). Shh expression was detected in surgical resection tissues of Barrett's metaplasia, but not in normal esophageal epithelium (35). The tissues of Barrett's metaplasia also demonstrated stromal expression of BMP4, a known target gene of Shh (35). In a mouse model of reflux esophagitis and Barrett's esophagus, Shh was expressed in the esophageal squamous cells prior to the development of intestinal metaplasia (35). These findings suggest that activation of the Shh-BMP-4 developmental signaling pathway by gastroesophageal reflux may contribute to the development of Barrett's metaplasia. While CDX2 expression is likely to play a role in the formation of Barrett's metaplasia, CDX2 alone is not enough to induce and intestinal-like phenotype in esophageal squamous cells (36). However, CDX2 is not the only transcription factor induced in epithelial cells by BMP-4 that has been linked with intestinal-like development. SOX-9 is a transcription factor that localizes to potential stem cells in intestinal crypts (35). Sox9 has recently been shown to be induced by BMP-4 in esophageal squamous cells and it has been implicated in the formation of Barrett's metaplasia (35). Like Shh, SOX9 expression has been detected in surgical resection tissues of Barrett's metaplasia, but not in normal esophageal squamous epithelium (35). Activation of downstream BMP-4 signaling pathways in esophageal squamous cells *in vitro* increased nuclear expression of SOX9 in the esophageal squamous cells (35). Using a novel *in vivo* transplant culture system, Wang et al. demonstrated that expression of Shh by esophageal squamous epithelial cells increased stromal expression of BMP-4 which in turn increased squamous epithelial cell expression of SOX9 and the columnar genes CK8 and 18 (35). These data suggest that activation of the Shh-BMP4-SOX9 pathway may also play a role in the formation of Barrett's metaplasia.

Emerging Concepts in Screening for Barrett's Esophagus

Data such as these suggest that it may be possible to use molecular markers to detect those patients predisposed to Barrett's esophagus. A number of studies, in addition to the ones noted above, have identified differences in genes involved in proliferation between esophageal squamous epithelium from patients with GERD who develop Barrett's esophagus and those from patients who heal their esophagitis by squamous cell regeneration. For example in squamous epithelium from GERD patients who do not develop Barrett's esophagus, there are low baseline levels of phospho-ERK (a pro-proliferative enzyme) and elevated levels of DKK1/4 (37; 38). In contrast, squamous epithelium from patients with Barrett's esophagus shows increased basal phospho-ERK and expression of an inhibitory form of phospho-MEK (37; 39). In addition to differences in proliferation markers, squamous epithelium from patients with BE also show differences in phenotypic markers such as Cdx2, as noted above, and the Das antigen, a

marker of Barrett's cells (33) (40). So perhaps in the future, it will be possible to use molecular expression profiles to distinguish the esophageal squamous mucosa of individuals who will develop Barrett's esophagus so we can target those patients for endoscopic screening and intensive anti-reflux therapy.

Emerging Concepts in the Treatment of Barrett's Esophagus

Investigators have used a number of endoscopic techniques to ablate Barrett's metaplasia in an attempt to eliminate the cancer risk. There are thermal modalities that use laser irradiation, multipolar electrocoagulation, argon plasma coagulation, or radiofrequency energy to burn away the metaplastic Barrett's mucosa (8). There has been intense interest in radiofrequency ablation (RFA) that uses a balloon-based device to deliver radiofrequency energy to the esophageal mucosa. This system is designed to inflict a uniform, circumferential thermal injury to the mucosa. Following RFA, patients are then put on PPI therapy and the epithelium heals with the formation of neo-squamous epithelium. A recent randomized, sham-controlled trial of RFA for dysplasia in Barrett's esophagus, in which our group at the VA participated, has spurred great interest in using RFA. In this study, Shaheen et al. randomized 64 patients with low grade dysplasia and 63 patients with high grade dysplasia in Barrett's esophagus in a 2:1 fashion to receive either real ablation or sham ablation (9). An intention to treat analysis showed that 81% of patients with high grade dysplasia who received RFA had no dysplasia detected at one year, compared to only 19% of patients in the sham group. For low-grade dysplasia the results were even better-91% of the ablated patients had no dysplasia detected at one year, compared to 23% in the sham ablation group. RFA also prevented progression of neoplasia from low grade to high dysplasia or from high grade dysplasia to cancer. Only 3.6% of the ablated patients had progression of neoplasia at one year compared to 16.3% of the sham group. And only 1.2% of the ablated patients had progression to cancer by one year compared to 9.3% of those in the sham group. There were 8 complications in 84 patients for a total complication rate of 9%. This included 5 esophageal strictures that were relatively easy to treat, one minor bleed, and two hospitalizations for chest pain. Although this is a fairly good safety record, it is important to note that the complication rate was not 0.

One question that comes up is once the Barrett's epithelium is replaced by neosquamous epithelium after RFA, is how long does it last? Does the Barrett's epithelium come back, and if so when? In one study by Fleischer et al., the investigators report that 46 of 50 patients (92%) with non-dysplastic Barrett's mucosa eradicated by RFA had no recurrence of the Barrett's mucosa at 5 years (41). However, the original study had 70 patients, only 60 who were eligible for the follow up study, and only 50 of those participated in the follow up study at 5 years (42). So if an intention to treat (ITT) analysis is applied to these data, then only 46 of 70 or 66% of patients had complete eradication of Barrett's mucosa at 5 years, which is a much less impressive rate than the 92% (41; 42). Moreover, there are also problems with the report that the durability of response is out to 5 years. All 50 patients in the follow up study had complete eradication of the Barrett's mucosa documented at 2.5 years after the first ablation procedure (41; 42). But to achieve eradication at the 2.5 year endpoint required a mean of 1.5 circumferential and 1.9 focal ablations AFTER the initial one. That means each patient had a mean of 3 separate ablation procedures to achieve complete eradication. During the subsequent 2.5 years, 8% of the patients had reappearance of Barrett's metaplasia that was again eliminated with a repeat focal ablation suggesting that the reversal of Barrett's mucosa by RFA does not last out to 5 years (41). In fact, these data suggest that patients will need regular endoscopic surveillance after ablation to identify and retreat recurrent Barrett's epithelium.

Based on these data the recent AGA Technical Review on the Management of Barrett's Esophagus has a number of suggestions regarding the use of RFA for patients with Barrett's esophagus (20). For patients with Barrett's esophagus and *no dysplasia*, the data from the Shaheen trial and others suggest that you can achieve reversion to normal-appearing squamous epithelium in over 97% of cases (9) (42). However the durability of this response is not known and a high rate of recurrent metaplasia has been reported, requiring patients to undergo continued endoscopic surveillence and repeat ablation. In addition, the goal of ablation is to prevent cancer and for patients who have *no dysplasia* in Barrett's esophagus, the risk of cancer is small, only .5% per year. Finally, there are no data that ablation protects against cancer development in these patients. Thus for patients who have Barrett's esophagus *without dysplasia* RFA is not recommended at this time (20). For patients with Barrett's esophagus and *low grade dysplasia*, reports suggest reversion to normal-appearing squamous epithelium in over 90% of cases however the same concerns remain over the durability of this response. The difficulties in the histologic diagnosis of dysplasia have caused uncertainty in the estimated risk of cancer. To the best of our knowledge the risk lies somewhere between .5% per year for non-dysplastic Barrett's and 6.6 % per year for high grade dysplasia in Barrett's esophagus (20). Regardless of the risk, benefit in cancer prevention has yet to be demonstrated. So it is not clear whether the benefits of ablation in reducing cancer risk for patients who have Barrett's esophagus with *low grade dysplasia* outweigh the risks and expense of the procedure, thus routine clinical use is not recommended at this time (20). However, physicians may opt to use this therapy in individual, selected cases. Finally, for patients with Barrett's esophagus and *high grade dysplasia*, reports suggest eradication of high grade dysplasia in 81% of cases. Durability of this response again is not known, but at least the trial by Shaheen et al. found no return of high grade dysplasia out to one year (9). This trial also demonstrated a benefit in preventing cancer progression. Thus for patients with *high grade dysplasia* in Barrett's esophagus, RFA is a reasonable option particularly in individuals of advanced age or those with co-morbidities that render other therapies (i.e. surgery) hazardous (20).

Conclusion

Gastroesophageal reflux disease and its complication of Barrett's esophagus are major risk factors for esophageal adenocarcinom, a lethal tumor whose incidence continues to increase in the US. Despite advances in endoscopic technology, surveillance strategies for early detection of these tumors remain largely ineffective, likely due the uncertainty regarding the individuals at risk for Barrett's esophagus. There is great potential clinical importance in elucidating the molecular pathways involved in the pathogenesis of GERD and Barrett's esophagus. These studies could identify molecular targets for therapies designed to prevent Barrett's metaplasia. Such therapies might be used for at least two important clinical purposes: 1) To prevent Barrett's esophagus from developing in patients with GERD and 2) To prevent Barrett's esophagus from re-developing in patients who have had endoscopic ablation of Barrett's epithelium. Prevention of the initial development of Barrett's metaplasia would virtually eliminate the risk of esophageal adenocarcinoma and would obviate the great expense and inconvenience of endoscopic surveillance for Barrett's esophagus. There is also burgeoning clinical interest in the use of radiofrequency ablation to eliminate the cancer risk of Barrett's esophagus, and such ablation can be cost-effective only if Barrett's metaplasia does not re-develop after the procedure. Clinicians should stay tuned as exciting new developments in this area are on the horizon.

Reference List

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