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MECHANISMS OF GASTROINTESTINAL, PANCREATIC AND LIVER DISEASES

Barrett's esophagus

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Abstract

Barrett's esophagus is an acquired metaplastic abnormality in which the normal stratified squamous epithelium lining of the esophagus is replaced by an intestinal-like columnar epithelium. While in itself a benign and asymptomatic disorder, the clinical importance of this relatively common condition relates to its role as a precursor lesion to esophageal adenocarcinoma, the incidence of which has dramatically increased in Western populations in recent years. Although known to arise as a consequence of chronic gastroesophageal reflux, the cellular and molecular mechanisms underlying development Barrett's esophagus and its progression to cancer remain unclear.

Barrett's esophagus is an acquired metaplastic abnormality in which the normal stratified squamous epithelium of the esophagus is replaced by an intestinal-like columnar epithelium containing goblet cells (intestinal metaplasia) (Fig. 1). The condition is widespread and confers upon sufferers a 100-fold increased risk of developing esophageal adenocarcinoma (EAC). The progression of Barrett's esophagus to EAC is a multistep process in which the metaplastic epithelium is thought to sequentially develop low-grade dysplasia (LGD), high-grade dysplasia (HGD), early EAC, and eventually, invasive carcinoma.[1,2]

Prevalence

During the last three decades, there have been rapid increases in the incidence of EAC in most Western populations.[3–7] While the increases in EAC incidence are undeniable, there is considerably less certainty about the occurrence of Barrett's esophagus within the population, let alone whether there have been changes over time. This is because Barrett's esophagus is often asymptomatic and definitive diagnosis requires access to specialized investigations (upper gastrointestinal endoscopy and histological confirmation). Access to endoscopy is far from universal, even for symptomatic patients, and thus, analyses of routinely-recorded hospital or pathology data are unlikely to estimate the true prevalence of Barrett's esophagus. Moreover, the cases identified in such datasets are likely to differ in terms of demographic and health characteristics from people living with undiagnosed Barrett's esophagus. Bearing these limitations in mind, the epidemiological data show a 2:1 male predominance among diagnosed cases,[8] with the typical age of diagnosis in the 50–59 years age group. Prevalence surveys in a multiracial setting suggest a higher prevalence of Barrett's esophagus in whites than blacks, Asians, or Hispanics.[9]

To overcome the influence of detection bias in estimating prevalence, investigators have conducted autopsy studies[10] or have systematically performed upper gastrointestinal endoscopy on patients referred for other investigations.[11] Such studies have estimated the prevalence of Barrett's esophagus in people without symptoms of reflux to range between 0.4% and 6%. Arguably, the most reliable estimates of Barrett's esophagus prevalence stemmed from an endoscopic survey of two communities in northern Sweden, in which a representative sample of 1000 local residents underwent endoscopy. Of these, 10.3% had columnar-lined esophagus on endoscopic visualization, and 1.6% had histologically-confirmed Barrett's esophagus.[12]

Several studies from different populations around the world have documented rapid increases in the diagnosis of Barrett's esophagus during recent decades.[13–15] While some of the increase in Barrett's esophagus is undoubtedly due to more widespread access to endoscopic services and higher rates of esophageal biopsy pathology, this does not appear to account for all of the increase in this condition,[13,14] and it is widely held that there has been a real increase in the incidence of Barrett's esophagus. The question arises as to what causes this condition.

Pathogenesis

The pathogenesis of Barrett's esophagus is poorly understood. Clinically, Barrett's esophagus is associated with long-standing,

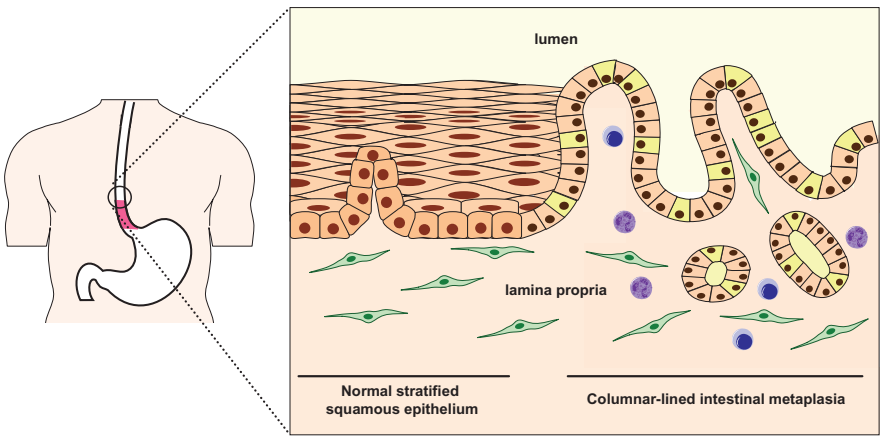

Figure 1 Structure of the luminal lining of the normal esophagus and Barrett's esophagus. Luminal surface of the esophagus is normally lined by a highly-organized stratified squamous epithelium. There is a single layer of basal cells that adhere to the basement membrane, followed by multiple layers of progressively flattened, differentiated squamous cells. Underlying lamina propria contains stromal cells (e.g. fibroblasts: green) and invaginates into the epithelium at regular intervals, producing tall papillary structures. In Barrett's esophagus, which invariable occurs in the distal third of the esophagus, the complex multilayered structure of the normal esophagus is replaced by a single-layered, intestinal-like columnar epithelium containing goblet cells (yellow). Barrett's metaplasia is also characterized by the presence of columnar-lined mucus-secreting glands and inflammatory cells (e.g. mononuclear cells: blue; neutrophils: purple).

symptomatic gastroesophageal reflux disease (GERD). Patients who reflux both gastric acid and duodenal contents (bile acids and pancreatic enzymes) have been found to have a higher prevalence of Barrett's esophagus than patients who reflux gastric juice alone.[16] This requirement for both acid and bile has been confirmed in animal models where, in the absence of gastroesophageal reflux, epithelial damage to the esophageal lining regenerated as squamous epithelium while, in the presence of reflux, regeneration resulted in columnar epithelium.[17] However, only 5–10% of patients with chronic reflux develop Barrett's esophagus, indicating that other genetic and/or environmental factors must also be involved.

Factors associated with increased risk

The risk factors for EAC have been elucidated with remarkable consistency by large-scale studies around the world (Table 1); however, identifying the risk factors for Barrett's esophagus has presented particular challenges since the patients who come to medical attention ("cases") are likely to differ from those who remain undiagnosed in the general population. Without careful consideration, a study comparing identified "cases" with "controls" (however defined) might spuriously identify factors associated with detection as being causal. For this reason, investigators have often chosen several different groups of comparators (e.g. patients undergoing endoscopy who do not have Barrett's esophagus; population controls) with the aim of teasing out detection factors from causal factors.[18]

There is general agreement that chronic reflux of gastric acid into the lower esophagus is the principal cause of Barrett's esophagus.[1] Studies comparing cases to population controls have typically reported 10-fold or greater relative risks for Barrett's esophagus associated with frequent symptoms of reflux.[18–21] When compared with other patients undergoing endoscopy, however, the

Table 1 Summary of risk factors for Barrett's esophagus

Risk factor	Barrett's esophagus	
	Direction	Agreement
Sex	Male : female = 2:1	†††
Ethnicity	Caucasian	†††
Socioeconomic status	+	Conflicting
Gastroesophageal reflux	+ + +	†††
Adiposity		
Body mass index	+	Conflicting
Central adiposity	+ +	†††
Physical activity	No data available	
Tobacco smoking	+	Conflicting
Total alcohol	0	†
Liquor	+	Conflicting
Beer	0	††
Wine	–	Conflicting
Fruits and vegetables	– –	††
Antioxidants	–	††
Dietary fat	+	††
Lower esophageal sphincter relaxing medications	Insufficient data available	
Proton pump inhibitors	No data available	
Non-steroidal anti-inflammatory drugs	Insufficient data available	
Helicobacter pylori	–	†

Note: 0, no association; +, weak-positive association; + +, moderate-positive association; + + +, strong positive association; –, weak-negative association; – –, moderate-negative association; †, weak agreement between studies; ††, moderate agreement between studies; †††, strong agreement between studies.

associations are attenuated substantially, and the data suggest that Barrett's esophagus patients have only slightly more frequent symptoms of reflux than GERD patients.[18,20,22]

Obesity has been strongly implicated as a risk factor for EAC with twofold–threefold increased risks for those with a body mass index (BMI) $\geq$30 kg/m^2;[23–26] however, associations with Barrett's esophagus have been inconsistent. This might reflect, at least partly, the choice of control groups in different studies. In a recent meta-analysis, Cook *et al*.[27] concluded that there was no association between BMI and Barrett's esophagus when the control group comprised GERD patients, but there was a positive association when cases were compared to population controls. Even so, population studies have suggested no more than a 50% increased risk of Barrett's esophagus associated with a BMI $\geq$30 kg/m^2,[18,20,28–30] considerably lower than risks observed for EAC. There is increasing evidence that the distribution of body fat is a more important risk factor for Barrett's esophagus risk than BMI. Strong associations between measures of central adiposity (including waist circumference and waist–hip ratio) and risk of Barrett's esophagus have been reported by several studies,[28,29] with the inference being that visceral fat is responsible for driving the association. This pattern of fat deposition is more common among men, and produces higher levels of obesity-related cytokines (such as leptin and low adiponectin) than the subcutaneous distribution of fat commonly observed in women. Because leptin is upregulated in obesity and has been shown to promote cellular proliferation in EAC cells *in vitro*, at least two studies have investigated its possible role in Barrett's esophagus. While both studies observed increased risks of Barrett's esophagus associated with higher leptin levels, one reported a stronger effect in men,[15] the other in women.[31] More data from larger studies are required to resolve whether leptin truly mediates the risk of Barrett's esophagus.

Most population-based studies,[18,20,29] but not all,[19,21] have reported approximately twofold increases in the risk of Barrett's esophagus associated with having ever smoked, although the risk of Barrett's esophagus does not increase in a dose-dependent manner with cumulative smoking exposure. It has been suggested that smoking and reflux might have synergistic effects on increasing the risk of Barrett's esophagus,[20] although this has not been observed in all studies. Well-conducted epidemiological studies find no evidence that alcohol intake increases the risk of Barrett's esophagus; two recent studies have suggested an inverse association with wine consumption.[32,33]

Case reports and pedigree studies suggest a heritable component to Barrett's esophagus, albeit with complex and variable expression.[34,35] Estimates of the prevalence of familial Barrett's esophagus vary; one large series reported a confirmed family history of Barrett's esophagus in 6% of probands;[36] another reported a prevalence of 24%.[37]

Factors associated with decreased risk

Serological evidence of infection with *Helicobacter pylori* has been identified consistently to confer reduced risks of EAC;[38] however, the evidence for Barrett's esophagus is less clear. A recent meta-analysis of 12 studies reported non-significantly reduced risk of Barrett's esophagus associated with *Helicobacter pylori* infection (odds ratio [OR]: 0.74, 95% confidence interval [CI]: 0.40–1.37), although subgroup analyses have suggested a significantly reduced risk in those studies comparing Barrett's esophagus cases to endoscopically-normal controls (OR: 0.50, 95% CI: 0.27–0.93).[39] Since that meta-analysis was published, two high-quality, population-based studies from California, USA[40] and Ireland[41] have reported significantly reduced risks of Barrett's esophagus associated with *Helicobacter pylori* infection (California OR: 0.42, 95% CI: 0.26–0.70; Ireland OR: 0.41, 95% CI: 0.27–0.62). Moreover, both studies observed reduced risks even after controlling for reflux symptoms, suggesting that not all of the "protective" effects could be explained simply by reduced gastric acid production.

Other factors that have been associated with reduced risks of developing Barrett's esophagus include frequent use of aspirin or non-steroidal anti-inflammatory drugs (NSAIDs),[42] dietary factors (including high intakes of fiber, fruit and vegetables, and meat),[43] and nutrients (including high intakes of vitamin C, beta-carotene, and vitamin E).[44] There is speculation, but limited evidence, that acid-suppressant medications might reduce the progression of Barrett's esophagus to cancer,[45] but whether they prevent the development of Barrett's esophagus in the first place is unknown.

Cellular origin

The cellular origin of the columnar cells of Barrett's esophagus is also not clear. Early suggestions that Barrett's metaplasia was the result of the migration of gastric columnar cells from the gastroesophageal junction have been largely discounted by animal studies.[46] It is now widely accepted that the columnar cells arise from within the esophagus, but there are several potential sources (Fig. 2). For example, they could arise as the result of a change in the stem cells responsible for the constant replenishing of the epithelial cells of the esophageal lining, such that they are reprogrammed to produce columnar, rather than squamous, cells. Some studies suggest that the stem cells of the esophageal epithelium reside in the basal layer, possibly in the intrapapillary regions.[47–49] Other studies suggest that a population of esophageal stem cells, and the source of metaplastic tissue in Barrett's esophagus, reside in the submucosal esophageal gland.[50–52] These glands are connected to the surface by a cuboidal cell-lined duct that penetrates the epithelium and opens into the esophageal lumen. It is hypothesized that pluripotent stem cells located distally within the duct lining become exposed following erosive esophagitis resulting from chronic reflux and promote the differentiation into intestinal-type columnar cells that migrate out to repopulate the injured epithelium. It has also been reported that bone marrow-derived stem cells contribute to the metaplastic tissue in a rat model of Barrett's esophagus.[53]

Alternatively, rather than an abnormality of stem cells, the acidic environment created by chronic reflux might induce transdifferentiation through an epigenetic effect on post-mitotic cells. During development, the esophagus is initially lined by a columnar-type epithelium that is replaced by the mature squamous epithelium during late embryogenesis through transdifferentiation.[54–56] This suggests the possibility that the columnar cells that characterize Barrett's metaplasia might result from a reversal in developmental programming. Consistent with this idea, the *in vitro* treatment of esophageal squamous cells with acid and bile can lead to the expression of columnar and/or intestinal cell markers.[57–60]

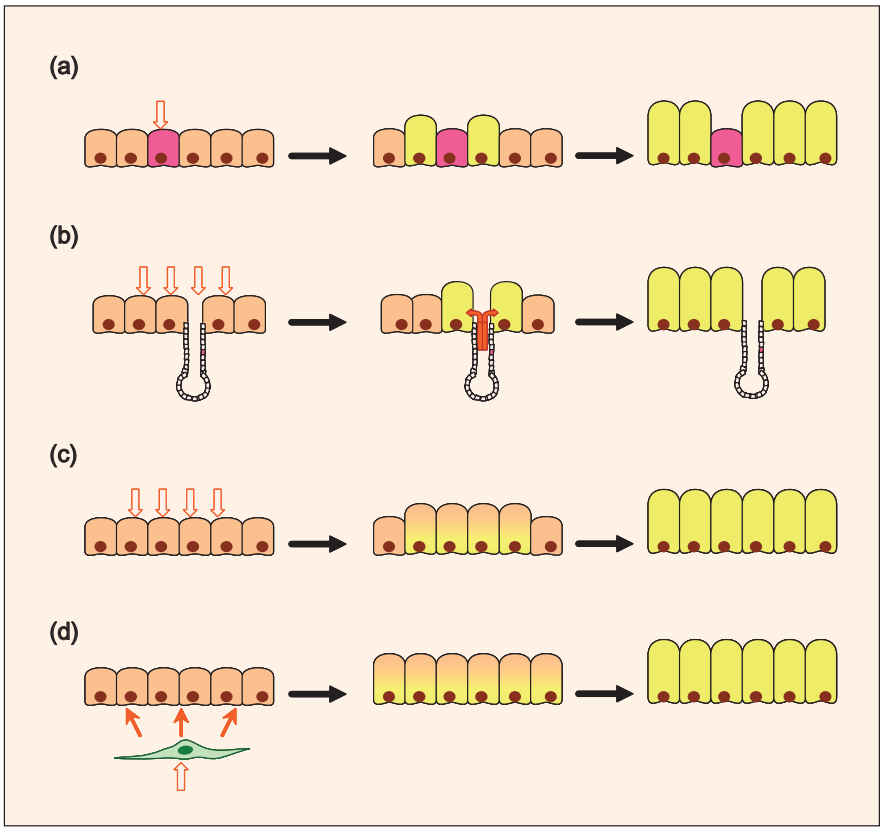

Figure 2 Hypotheses for the origin of columnar cells in Barrett's esophagus. Columnar cells that characterize Barrett's esophagus might arise (a) by the reprogramming of a stem cell situated in the basal layer of the normal squamous epithelium, (b) by the migration and differentiation of cells from the lining of the esophageal gland duct, (c) through the transdifferentiation of squamous cells in the normal esophageal epithelium, or (d) as the result of changes in regulatory signals from the stromal compartment. Development of Barrett's esophagus is depicted as the progression from a normal squamous epithelium (left) to a columnar epithelium (right). Illustrated are basal cells of the squamous epithelium (orange), intestinal-type columnar cells (yellow), stem cells (pink), and stromal fibroblasts (green). Open arrows indicate acid/bile insult.

A third possibility is that Barrett's metaplasia arises not from direct effects on the epithelial cells, but indirectly as a consequence of changes (mutational and/or environmental) in the stromal cells (e.g. myofibroblasts and inflammatory cells) of the submucosa. Cytokines and other regulatory signals emanating from the stromal cells could potentially influence the differentiation and development of cells within the epithelial layer.[61] It has also been suggested that the columnar epithelium of Barrett's esophagus might arise directly from stromal cells via a mesenchymal-to-epithelial transition.[50]

Molecular basis

Barrett's esophagus usually develops in the context of chronic gastroesophageal reflux. Presumably, the repeated exposure to acid and bile in the refluxate induces tissue injury in the lower esophagus, and the intestinal metaplasia that forms during the healing process likely reflects an adaptive response in which the damaged mucosa is replaced with a more acid- and bile-resistant epithelium. The prevailing view is that acid and bile in the refluxate, either directly or indirectly, induces genetic and/or epigenetic changes that lead to the onset of Barrett's esophagus and its progression to cancer.

Genetic abnormalities

Multiple genetic changes are detectable in Barrett's esophagus. Whole-genome studies have demonstrated that the majority of Barrett's esophagus samples show some level of chromosomal instability, as characterized by copy number gains, copy number losses, and the loss of heterozygosity (LOH).[62–64] These changes increase in frequency and size as the condition progresses, with single nucleotide polymorphism (SNP) array analyses suggesting that genomic abnormalities increase from involving less than 2% of the genome in early-stage Barrett's metaplasia to over 30% in late-stage Barrett's esophagus.[63] The most frequent change seen is loss of the short arm of chromosome 9, including 9p21.3 (CDKN2A/p16). Other common abnormalities in early-stage Barrett's esophagus include copy loss on 3p across the *FHIT* gene locus (3p59.8–60.6) and 16q, across the WWOX locus

(16q77.3).[63] A variety of somatic genetic alterations usually associated with cancer, including the loss of p53, adenomatous polyposis coli (APC), and Rb (retinoblastoma protein), and the overexpression of cyclin D1, Bcl2, and SRC kinase, are also readily detectable in Barrett's metaplasia tissue.[65] However, there is little evidence that these events have a direct role in the development of Barrett's esophagus itself, and it is likely that many are actually early events in the progression of Barrett's metaplasia to dysplasia and EAC.

Similarly, gene array studies have reported many genes that are differentially expressed between Barrett's esophagus and normal esophageal mucosa[66,67] but the problem is in distinguishing between those changes that simply reflect the phenotypic differences between squamous and columnar cells and those that are actually responsible for driving the transdifferentiation process.

Cdx

Intuitively, the mechanism(s) directly driving transdifferentiation is/are likely to involve important transcriptional regulators, such as the homeobox genes, a family of DNA-binding proteins that play a crucial role in tissue patterning and cell fate determination. Cdx1 and Cdx2 are intestinal-specific transcription factors that are thought to direct the development and differentiation of the columnar epithelium in the intestine,[68,69] and there is increasing evidence to suggest they might have a role in the development of Barrett's esophagus. Although neither is expressed in the normal esophagus or stomach, both are highly expressed in regions of intestinal metaplasia in these tissues.[70–73] Strikingly, the transgenic expression of Cdx1 or Cdx2 in the stomach leads to the development of intestinal metaplasia in mice,[74–76] while the loss of Cdx2 in intestinal tissue leads to the formation of stratified squamous epithelium similar to that found in the esophagus.[77] Furthermore, chronic exposure to acid induces the expression of Cdx2 in normal mouse esophageal cells.[60] While these data strongly support Cdx1 and Cdx2 as likely candidate genes involved in the development of Barrett's esophagus, attempts to demonstrate this have not been successful, suggesting that these genes alone are insufficient to drive the generation of a columnar phenotype in the esophagus (Dr Daniel Croagh & Associate Professor Wayne Phillips, unpublished data).

Hedgehog signaling

The hedgehog (Hh) signaling pathway is critical for normal gut development, and thus represents another potential candidate as a molecular mediator of Barrett's esophagus. Hh signaling is crucial to the development of columnar epithelium in the gastrointestinal tract, including the early esophagus.[78,79] Hh signaling is extinguished during the transition of the esophageal epithelium from the primitive columnar cells that characterize the embryonic esophagus to the stratified squamous lining of the adult organ.[78] However, recent studies have shown that while the normal squamous epithelium of the esophagus does not express the Hh ligands Sonic hedgehog and Indian hedgehog, both are markedly upregulated in Barrett's esophagus[80] and in acid-treated esophageal squamous epithelial cells.[80,81] Consistent with the activation of Hh signaling in Barrett's esophagus, the Hh target genes *Ptch1* and *Bmp4* were found to be expressed in the stromal compartment associated with Barrett's esophagus, but not in the stroma underlying normal squamous epithelium.[80] Furthermore, BMP4 (bone morphogenic protein 4) was shown to induce the expression of SOX9, a transcription factor known to upregulate the expression of *DMBT1* (*Deleted in Malignant Brain Tumors 1*), a gene linked to the induction of columnar epithelial differentiation, thus providing a potential mechanism by which Hh signaling could mediate the development of intestinal metaplasia in the esophagus.

Biomarkers

The search for biomarkers that can identify or predict the progression of Barrett's esophagus to EAC has been motivated, in particular, by the limitations of endoscopic surveillance programs. Very many potential Barrett's esophagus biomarkers and biomarker studies have been reported. Most studies have included cross-sectional convenience samples of tissues from heterogeneous patients, and have inadequate patient numbers, inadequate follow-up durations, and fail to show reproducibility. Many studies also include tissues with different stages of Barrett's esophagus from the same Barrett's esophagus segment, despite a possible field effect in which, for example, even squamous esophageal mucosa from patients with EAC differs from normal epithelium from patients without EAC.[66,82] The molecular signature of normal squamous esophageal epithelium identifies the presence of a field effect and can discriminate between patients with Barrett's esophagus and patients with Barrett's-associated adenocarcinoma.[83] This review focuses on those biomarkers for which there are stronger data, preferably from prospective studies in which the same patient cohort is followed with sequential biopsies or other specimens.

The development and progression of Barrett's esophagus results from the evolution of a clone of cells along one of multiple complex pathways of increasing genetic and epigenetic abnormality.[84] Flow cytometric, cytogenetic, comparative genomic hybridization, and other studies have shown that aneuploidy (an increase or decrease in the cell chromosome number by one or more chromosomes), other large chromosomal losses resulting in loss of a gene copy (LOH, allelic loss) and cell-cycle alterations are more frequent at higher grades of dysplasia. Aneuploid cell populations are found in approximately two-thirds of patients with HGD and in approximately 90% of those with EAC. Increased proportions of cells in the S and G2 phases of the cell cycle are also frequently present in dysplastic tissues.

A series of prospective studies from the Fred Hutchinson Cancer Research Center, Seattle, USA, have demonstrated the predictive potential of chromosome analyses. Galipeau *et al.* reported in 2007 on 243 patients with Barrett's esophagus in whom baseline analysis at study entry included aneuploidy and tetraploidy detection, as well as assessment of the tumor-suppressor genes *p16/CDKN2A* and *p53*.[85] Tumor-suppressor genes, like other genes, might be inactivated by mutation, by loss of a gene copy (LOH), or by the epigenetic suppression of gene expression by DNA hypermethylation, which involves the abnormal addition of methyl (CH3) groups to cytosine bases at particular sites (CpG dinucleotides) in gene promoter regions. At 10 years' follow up, all biomarkers, except p16 mutation and methylation, were significantly associated with the risk of EAC development. The relative risk of developing EAC at 5 years in those with baseline 9pLOH and

17pLOH and a DNA content abnormality was 79.1%, compared to no cases of EAC development in patients with none of these abnormalities at baseline.[85]

Clinical introduction of these highly-promising biomarkers requires further clinical trials. It also needs to be shown that the laboratory methods can be performed widely in the formalin-fixed, paraffin-embedded (FFPE) tissues that are stored in pathology departments worldwide. Some methods of ploidy assessment require large amounts of FFPE tissue.[86] Fluorescence *in situ* hybridization (FISH) requires less tissue, but prospective trials are required before a FISH-based biomarker approach should be considered for clinical practice.[87,88]

The study above by Galipeau *et al*. also indicated that multiple chromosomal instability biomarkers can identify patients in whom aspirin and other NSAIDs reduce progression risk.[85] Cyclooxygenase 2 (COX-2) expression is increased in Barrett's esophagus and EAC tissues, and the use of aspirin and NSAIDs is associated with reduced esophageal cancer risk in population-based studies. Although a celecoxib COX-2 inhibition trial failed to show a benefit in terms of prevention of dysplasia to EAC,[89] only 100 patients were included, and biomarkers were assessed as surrogate end-points, rather than to guide patient selection. A chemoprevention trial using a proton pump inhibitor (PPI), with or without aspirin, will report interim results in 2011.[90]

In contrast to the studies above, a case-control study of 27 patients who progressed to HGD or EAC, and an equal number without progression, found that in patients with LGD, aneuploidy measured by flow cytometry was not a significant predictive factor, but p53 and Ki67 protein overexpression measured by immunohistochemistry were important factors for neoplastic progression.[91] An appealing aspect of these results is the simple methods involved, but the study included few patients and was retrospective.

The potential for assessing DNA methylation markers alone has been shown by several longitudinal studies, including one retrospective multicentre, double-blinded validation study of eight methylation markers from the Meltzer laboratory at Johns Hopkins University (Baltimore, MD, USA).[92] Based on results from a cross-sectional study, Wang *et al*. retrospectively compared the *p16* and *APC* methylation status of seven patients who progressed to HGD or EAC with 50 non-progressors.[93] Again, patient numbers were very limited, but nevertheless, none of the patients without hypermethylation of both genes at baseline progressed to HGD or cancer.[93] APC is another potential blood-based biomarker. In one study, CpG island promoter region hypermethylation of the *APC* gene was detectable in the plasma of 14 (26%) of 54 patients with EAC, but was not detected in patients without cancer, including patients with Barrett's esophagus (0/45 patients).[94]

Genes that have markedly upregulated or down-regulated mRNA expression in either HGD or EAC tissues show promise as part of a panel of informative genes. In some studies, the Barrett's esophagus histopathological diagnosis can be predicted in almost all patients by measuring mRNA expression levels.[66,94] Obstacles to this approach include the wide range of expression values, sometimes with overlap between all stages for individual genes, and the current limited availability of accurate quantitative mRNA expression measurement in FFPE tissues outside of research laboratories. Sequential studies are needed to test the accuracy of expression panels.

Very high telomerase mRNA expression levels are found in EAC, but not Barrett's esophagus tissues.[82] Risques *et al*. prospectively measured telomere length in baseline blood samples in a cohort of 300 patients with Barrett's esophagus followed up for a mean of 5.8 years. Leukocyte telomere length predicted the risk of EAC independently of smoking, obesity, and NSAID use (hazard ratio: 4.18; 95% CI: 1.60–10.94; $P = 0.004$).[95]

MicroRNA (miRNA) are small, "non-coding sequence" RNA molecules containing 21–22 nucleotides that regulate gene expression at the post-transcriptional level. Alterations in miRNA levels are associated with dysplasia and cancer, can regulate oncogenes, and have oncogenic capacity.[96] The potential role of miRNA as biomarkers has been shown by a study that found miR-21 (reported as overexpressed in several solid tumors) was overexpressed in Barrett's esophagus and EAC relative to squamous epithelium, whereas miR-143, miR-145, and miR-215 were underexpressed in EAC relative to non-neoplastic Barrett's esophagus.[97]

The important clinical issue of estimating the effectiveness of endoscopic ablation has been studied by using molecular markers as surrogate end-points for clinical outcomes, since it is likely to be many years before the clinical outcomes are known. Comparing pre-radiofrequency ablation (RFA) Barrett's esophagus tissues with the neosquamous epithelium that replaces the Barrett's esophagus, Pouw *et al*. reported that Ki-67 and p53 protein expressions, aneuploidy involving chromosomes 1 and 9, and *p16* and *p53* LOH were all normalized in 22 patients with Barrett's esophagus containing early cancer and/or high-grade intra-epithelial neoplasia.[98] This study suggests that the post-RFA neosquamous epithelium is both morphologically and genetically similar to normal squamous epithelium, although further studies are required. Neosquamous epithelium from argon plasma coagulation-ablated patients has higher steady state levels of microRNA miR-143 than Barrett's esophagus does, with values being higher than in squamous epithelium taken from patients without Barrett's esophagus.[99]

Clinical management

Barrett's esophagus is the major risk factor for the development of EAC. Nearly 90% of people who develop advanced EAC die from this disease. Thus, early detection of cancer or prevention of progression from Barrett's esophagus are obvious strategies that should be considered in the clinical management of Barrett's esophagus.

Currently, the management of Barrett's esophagus focuses on treating reflux and managing the risk of cancer development. Reflux control is achieved by acid suppression with PPI medication or surgery (fundoplication). PPI are first-line treatment, with surgery undertaken for ongoing symptoms, despite adequate PPI therapy. While there have been isolated reports of Barrett's esophagus regressing following PPI therapy[100] and fundoplication,[101,102] neither form of antireflux therapy produces predictable regression, or reliably prevents cancer. Efforts have therefore focused on managing the risk of cancer development by surveillance and techniques that might reverse the disease.

Surveillance

Endoscopic surveillance is undertaken every 2 years, according to international consensus guidelines,[103] but if LGD is identified,

surveillance is usually shortened to six monthly. HGD or cancer develops in 0.2–2% of patients with Barrett's esophagus each year of surveillance follow up. The conversion rate varies across different studies, with many at the lower end of this range. This might be related to variable surveillance practices and different biopsy collection protocols, with many endoscopists collecting too few biopsies, but undertaking endoscopy too frequently.[104–106] A systematic approach to surveillance practice, and strict protocol compliance in collecting biopsies, increases the rate of detection of HGD and early cancer to more than 1%, while at the same time, reducing the actual number of endoscopy procedures per individual.[107]

Esophagectomy

Until recently, the clinical management of Barrett's esophagus entailed endoscopic surveillance and progression to esophagectomy if HGD or early cancer developed. However, the impact of new endoscopic treatments, such as ablation[108–110] and endoscopic mucosal resection (EMR),[111] has diminished the role of esophagectomy. Endoscopic treatment preserves an intact esophagus, and its uptake has been encouraged by the belief that esophagectomy is associated with a significant risk of perioperative death. However, esophagectomy for HGD tends to be undertaken in younger and "fitter" patients, and less extensive surgery is required than for advanced cancer.[112] This is associated with surgical mortality rates of less than 1%, and the cure rate is very high.[112]

Ablation

In patients in whom reflux has been managed successfully, the destruction (ablation) of metaplastic mucosa is usually followed by repopulation with a squamous epithelium.[102,108]

A range of endoscopic ablation techniques have been described, including photodynamic therapy, argon plasma coagulation, cryotherapy, and RFA. While these techniques differ somewhat in terms of the response to mucosal destruction, it appears that destruction of metaplastic mucosa in an acid-free environment is usually followed by regeneration with a histologically-normal (neo) squamous mucosa. While two randomized trials provide support for the idea that ablation can prevent malignancy,[113,114] there remains potential for malignancy to arise in areas of retained or buried columnar mucosa, and possibly even from within the neosquamous mucosa.[115,116] If ablation can be shown to sufficiently reduce the risk of cancer in a cost-effective manner, then the current paradigm of endoscopic surveillance might shift towards screening, followed by ablation of all identified Barrett's esophagus. Currently, however, there is no evidence to support such a strategy.

EMR

EMR is an alternative endoscopic approach in which the epithelium is excised, rather than ablated, thus allowing for a definitive histological diagnosis, while also potentially being curative. EMR excises a piece of mucosa that is approximately 1.5–2 cm in diameter. This approach can be used for the definitive treatment of some intramucosal cancers arising in Barrett's esophagus, or circumferentially, using multiple excisions to remove the entire segment of Barrett's metaplasia. Circumferential EMR resection is followed by esophageal stricture formation in 10–40% of patients.[117–119] These risks increase in proportion to the number of mucosal resections undertaken, and for this reason, EMR-based excision of the full segment of Barrett's esophagus is usually limited to shorter lengths.

Conclusions

Barrett's esophagus is an acquired metaplastic abnormality in which the normal stratified squamous epithelium lining of the esophagus is replaced by an intestinal-like columnar epithelium. Although known to develop as a consequence of chronic gastroesophageal reflux, the cellular and molecular mechanisms underlying the development of Barrett's esophagus and its progression to cancer remain unclear. A lack of effective biomarkers to predict the progression to EAC means that clinical management is focused on managing the risk of cancer by surveillance and ablation techniques aimed at eradicating the metaplastic tissue.

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References

1 Falk GW. Barrett's esophagus. *Gastroenterology* 2002; **122**: 1569–91.
2 Wild CP, Hardie LJ. Reflux, Barrett's oesophagus and adenocarcinoma: burning questions. *Nat. Rev. Cancer* 2003; **3**: 676–84.
3 Lord RV, Law MG, Ward RL, Giles GG, Thomas RJ, Thursfield V. Rising incidence of oesophageal adenocarcinoma in men in Australia. *J. Gastroenterol. Hepatol.* 1998; **13**: 356–62.
4 Botterweck AA, Schouten LJ, Volovics A, Dorant E, van-Den-Brandt PA. Trends in incidence of adenocarcinoma of the oesophagus and gastric cardia in ten European countries. *Int. J. Epidemiol.* 2000; **29**: 645–54.
5 el-Serag HB. The epidemic of esophageal adenocarcinoma. *Gastroenterol. Clin. North Am.* 2002; **31**: 421–40, viii.
6 Pohl H, Welch HG. The role of overdiagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence. *J. Natl. Cancer Inst.* 2005; **97**: 142–6.
7 Stavrou EP, McElroy HJ, Baker DF, Smith G, Bishop JF. Adenocarcinoma of the oesophagus: incidence and survival rates in New South Wales, 1972–2005. *Med. J. Aust.* 2009; **191**: 310–4.
8 Cook MB, Wild CP, Forman D. A systematic review and meta-analysis of the sex ratio for Barrett's esophagus, erosive reflux disease, and nonerosive reflux disease. *Am. J. Epidemiol.* 2005; **162**: 1050–61.
9 Abrams JA, Fields S, Lightdale CJ, Neugut AI. Racial and ethnic disparities in the prevalence of Barrett's esophagus among patients who undergo upper endoscopy. *Clin. Gastroenterol. Hepatol.* 2008; **6**: 30–4.

10 Cameron AJ, Zinsmeister AR, Ballard DJ, Carney JA. Prevalence of columnar-lined (Barrett's) esophagus. Comparison of population-based clinical and autopsy findings. *Gastroenterology* 1990; **99**: 918–22.
11 Rex DK, Cummings OW, Shaw M *et al*. Screening for Barrett's esophagus in colonoscopy patients with and without heartburn. *Gastroenterology* 2003; **125**: 1670–7.
12 Ronkainen J, Aro P, Storskrubb T *et al*. Prevalence of Barrett's esophagus in the general population: an endoscopic study. *Gastroenterology* 2005; **129**: 1825–31.
13 Prach AT, MacDonald TA, Hopwood DA, Johnston DA. Increasing incidence of Barrett's oesophagus: education, enthusiasm, or epidemiology? *Lancet* 1997; **350**: 933.
14 Conio M, Cameron AJ, Romero Y *et al*. Secular trends in the epidemiology and outcome of Barrett's oesophagus in Olmsted County, Minnesota. *Gut* 2001; **48**: 304–9.
15 Kendall BJ, Macdonald GA, Hayward NK *et al*. Leptin and the risk of Barrett's oesophagus. *Gut* 2008; **57**: 448–54.
16 Clark GW, Ireland AP, DeMeester TR. Dysplasia in Barrett's esophagus: diagnosis, surveillance and treatment. *Dig. Dis.* 1996; **14**: 213–27.
17 Koak Y, Winslet M. Changing role of *in vivo* models in columnar-lined lower esophagus. *Dis. Esophagus* 2002; **15**: 271–7.
18 Johansson J, Hakansson HO, Mellblom L *et al*. Risk factors for Barrett's oesophagus: a population-based approach. *Scand. J. Gastroenterol.* 2007; **42**: 148–56.
19 Anderson LA, Watson RG, Murphy SJ *et al*. Risk factors for Barrett's oesophagus and oesophageal adenocarcinoma: results from the FINBAR study. *World J. Gastroenterol.* 2007; **13**: 1585–94.
20 Smith KJ, O'Brien SM, Green AC, Webb PM, Whiteman DC. Current and past smoking significantly increase risk for Barrett's esophagus. *Clin. Gastroenterol. Hepatol.* 2009; **7**: 840–8.
21 Kubo A, Levin TR, Block G *et al*. Cigarette smoking and the risk of Barrett's esophagus. *Cancer Causes Control* 2009; **20**: 303–11.
22 Edelstein ZR, Bronner MP, Rosen SN, Vaughan TL. Risk factors for Barrett's esophagus among patients with gastroesophageal reflux disease: a community clinic-based case-control study. *Am. J. Gastroenterol.* 2009; **104**: 834–42.
23 Vaughan TL, Davis S, Kristal A, Thomas DB. Obesity, alcohol, and tobacco as risk factors for cancers of the esophagus and gastric cardia: adenocarcinoma versus squamous cell carcinoma. *Cancer Epidemiol. Biomarkers Prev.* 1995; **4**: 85–92.
24 Brown LM, Swanson CA, Gridley G *et al*. Adenocarcinoma of the esophagus: role of obesity and diet. *J. Natl. Cancer Inst.* 1995; **87**: 104–9.
25 Lagergren J, Bergstrom R, Nyren O. Association between body mass and adenocarcinoma of the esophagus and gastric cardia. *Ann. Intern. Med.* 1999; **130**: 883–90.
26 Whiteman DC, Sadeghi S, Pandeya N *et al*. Combined effects of obesity, acid reflux and smoking on the risk of adenocarcinomas of the oesophagus. *Gut* 2008; **57**: 173–80.
27 Cook MB, Greenwood DC, Hardie LJ, Wild CP, Forman D. A systematic review and meta-analysis of the risk of increasing adiposity on Barrett's esophagus. *Am. J. Gastroenterol.* 2008; **103**: 292–300.
28 Corley DA, Kubo A, Levin TR *et al*. Abdominal obesity and body mass index as risk factors for Barrett's esophagus. *Gastroenterology* 2007; **133**: 34–41.
29 Edelstein ZR, Farrow DC, Bronner MP, Rosen SN, Vaughan TL. Central adiposity and risk of Barrett's esophagus. *Gastroenterology* 2007; **133**: 403–11.
30 Jacobson BC, Chan AT, Giovannucci EL, Fuchs CS. Body mass index and Barrett's oesophagus in women. *Gut* 2009; **58**: 1460–6.
31 Thompson OM, Beresford SA, Kirk EA, Bronner MP, Vaughan TL. Serum leptin and adiponectin levels and risk of Barrett's esophagus and intestinal metaplasia of the gastroesophageal junction. *Obesity* 2010; **31**: 2204–11.
32 Kubo A, Levin TR, Block G *et al*. Alcohol types and sociodemographic characteristics as risk factors for Barrett's esophagus. *Gastroenterology* 2009; **136**: 806–15.
33 Anderson LA, Cantwell MM, Watson RG *et al*. The association between alcohol and reflux esophagitis, Barrett's esophagus, and esophageal adenocarcinoma. *Gastroenterology* 2009; **136**: 799–805.
34 di Pietro M, Fitzgerald RC. Barrett's oesophagus: an ideal model to study cancer genetics. *Hum. Genet.* 2009; **126**: 233–46.
35 Sun X, Elston R, Barnholtz-Sloan J *et al*. A segregation analysis of Barrett's esophagus and associated adenocarcinomas. *Cancer Epidemiol. Biomarkers Prev.* 2010; **19**: 666–74.
36 Chak A, Ochs-Balcom H, Falk G *et al*. Familiality in Barrett's esophagus, adenocarcinoma of the esophagus, and adenocarcinoma of the gastroesophageal junction. *Cancer Epidemiol. Biomarkers Prev.* 2006; **15**: 1668–73.
37 Chak A, Lee T, Kinnard MF *et al*. Familial aggregation of Barrett's oesophagus, oesophageal adenocarcinoma, and oesophagogastric junctional adenocarcinoma in Caucasian adults. *Gut* 2002; **51**: 323–8.
38 Islami F, Kamangar F. *Helicobacter pylori* and esophageal cancer risk: a meta-analysis. *Cancer Prev. Res. (Phila. Pa)* 2008; **1**: 329–38.
39 Wang C, Yuan Y, Hunt RH. *Helicobacter pylori* infection and Barrett's esophagus: a systematic review and meta-analysis. *Am. J. Gastroenterol.* 2009; **104**: 492–500, quiz 491, 501.
40 Corley DA, Kubo A, Levin TR *et al*. *Helicobacter pylori* infection and the risk of Barrett's oesophagus: a community-based study. *Gut* 2008; **57**: 727–33.
41 Anderson LA, Murphy SJ, Johnston BT *et al*. Relationship between *Helicobacter pylori* infection and gastric atrophy and the stages of the oesophageal inflammation, metaplasia, adenocarcinoma sequence: results from the FINBAR case-control study. *Gut* 2008; **57**: 734–9.
42 Anderson LA, Johnston BT, Watson RG *et al*. Nonsteroidal anti-inflammatory drugs and the esophageal inflammation-metaplasia-adenocarcinoma sequence. *Cancer Res.* 2006; **66**: 4975–82.
43 Kubo A, Block G, Quesenberry CP Jr, Buffler P, Corley DA. Effects of dietary fiber, fats, and meat intakes on the risk of Barrett's esophagus. *Nutr. Cancer* 2009; **61**: 607–16.
44 Kubo A, Levin TR, Block G *et al*. Dietary antioxidants, fruits, and vegetables and the risk of Barrett's esophagus. *Am. J. Gastroenterol.* 2008; **103**: 1614–23, quiz 24.
45 El-Serag HB, Aguirre TV, Davis S, Kuebeler M, Bhattacharyya A, Sampliner RE. Proton pump inhibitors are associated with reduced incidence of dysplasia in Barrett's esophagus. *Am. J. Gastroenterol.* 2004; **99**: 1877–83.
46 Gillen P, Keeling P, Byrne PJ, West AB, Hennessy TP. Experimental columnar metaplasia in the canine oesophagus. *Br. J. Surg.* 1988; **75**: 113–15.
47 Croagh D, Phillips WA, Redvers R, Thomas RJ, Kaur P. Identification of candidate murine esophageal stem cells using a combination of cell kinetic studies and cell surface markers. *Stem Cells* 2007; **25**: 313–18.
48 Seery JP. Stem cells of the oesophageal epithelium. *J. Cell Sci.* 2002; **115**: 1783–9.
49 Seery JP, Watt FM. Asymmetric stem-cell divisions define the architecture of human oesophageal epithelium. *Curr. Biol.* 2000; **10**: 1447–50.

50 Chang CL, Lao-Sirieix P, Save V, De La Cueva Mendez G, Laskey R, Fitzgerald RC. Retinoic acid-induced glandular differentiation of the oesophagus. *Gut* 2007; **56**: 906–17.
51 Coad RA, Woodman AC, Warner PJ, Barr H, Wright NA, Shepherd NA. On the histogenesis of Barrett's oesophagus and its associated squamous islands: a three-dimensional study of their morphological relationship with native oesophageal gland ducts. *J. Pathol.* 2005; **206**: 388–94.
52 Leedham SJ, Preston SL, McDonald SA *et al*. Individual crypt genetic heterogeneity and the origin of metaplastic glandular epithelium in human Barrett's oesophagus. *Gut* 2008; **57**: 1041–8.
53 Sarosi G, Brown G, Jaiswal K *et al*. Bone marrow progenitor cells contribute to esophageal regeneration and metaplasia in a rat model of Barrett's esophagus. *Dis. Esophagus* 2008; **21**: 43–50.
54 DeNardi FG, Riddell RH. The normal esophagus. *Am. J. Surg. Pathol.* 1991; **15**: 296–309.
55 Johns BA. Developmental changes in the oesophageal epithelium in man. *J. Anat.* 1952; **86**: 431–42.
56 Yu WY, Slack JM, Tosh D. Conversion of columnar to stratified squamous epithelium in the developing mouse oesophagus. *Dev. Biol.* 2005; **284**: 157–70.
57 Bajpai M, Liu J, Geng X, Souza RF, Amenta PS, Das KM. Repeated exposure to acid and bile selectively induces colonic phenotype expression in a heterogeneous Barrett's epithelial cell line. *Lab. Invest.* 2008; **88**: 643–51.
58 Burnat G, Rau T, Elshimi E, Hahn EG, Konturek PC. Bile acids induce overexpression of homeobox gene CDX-2 and vascular endothelial growth factor (VEGF) in human Barrett's esophageal mucosa and adenocarcinoma cell line. *Scand. J. Gastroenterol.* 2007; **42**: 1460–5.
59 Fitzgerald RC, Omary MB, Triadafilopoulos G. Dynamic effects of acid on Barrett's esophagus. An *ex vivo* proliferation and differentiation model. *J. Clin. Invest.* 1996; **98**: 2120–8.
60 Marchetti M, Caliot E, Pringault E. Chronic acid exposure leads to activation of the cdx2 intestinal homeobox gene in a long-term culture of mouse esophageal keratinocytes. *J. Cell Sci.* 2003; **116**: 1429–36.
61 Kosinski C, Stange DE, Xu C *et al*. Indian hedgehog regulates intestinal stem cell fate through epithelial–mesenchymal interactions during development. *Gastroenterology* 2010; **139**: 893–903.
62 Chaves P, Crespo M, Ribeiro C *et al*. Chromosomal analysis of Barrett's cells: demonstration of instability and detection of the metaplastic lineage involved. *Mod. Pathol.* 2007; **20**: 788–96.
63 Li X, Galipeau PC, Sanchez CA *et al*. Single nucleotide polymorphism-based genome-wide chromosome copy change, loss of heterozygosity, and aneuploidy in Barrett's esophagus neoplastic progression. *Cancer Prev. Res. (Phila. Pa)* 2008; **1**: 413–23.
64 Walch AK, Zitzelsberger HF, Bruch J *et al*. Chromosomal imbalances in Barrett's adenocarcinoma and the metaplasia–dysplasia–carcinoma sequence. *Am. J. Pathol.* 2000; **156**: 555–66.
65 Jenkins GJ, Doak SH, Parry JM, D'Souza FR, Griffiths AP, Baxter JN. Genetic pathways involved in the progression of Barrett's metaplasia to adenocarcinoma. *Br. J. Surg.* 2002; **89**: 824–37.
66 Brabender J, Marjoram P, Salonga D *et al*. A multigene expression panel for the molecular diagnosis of Barrett's esophagus and Barrett's adenocarcinoma of the esophagus. *Oncogene* 2004; **23**: 4780–8.
67 Greenawalt DM, Duong C, Smyth GK *et al*. Gene expression profiling of esophageal cancer: comparative analysis of Barrett's esophagus, adenocarcinoma, and squamous cell carcinoma. *Int. J. Cancer* 2007; **120**: 1914–21.
68 Freund JN, Domon-Dell C, Kedinger M, Duluc I. The *Cdx-1* and *Cdx-2* homeobox genes in the intestine. *Biochem. Cell Biol.* 1998; **76**: 957–69.
69 Lohnes D. The Cdx1 homeodomain protein: an integrator of posterior signaling in the mouse. *Bioessays* 2003; **25**: 971–80.
70 Bai YQ, Yamamoto H, Akiyama Y *et al*. Ectopic expression of homeodomain protein CDX2 in intestinal metaplasia and carcinomas of the stomach. *Cancer Lett.* 2002; **176**: 47–55.
71 Eda A, Osawa H, Satoh K *et al*. Aberrant expression of CDX2 in Barrett's epithelium and inflammatory esophageal mucosa. *J. Gastroenterol.* 2003; **38**: 14–22.
72 Lord RV, Brabender J, Wickramasinghe K *et al*. Increased CDX2 and decreased PITX1 homeobox gene expression in Barrett's esophagus and Barrett's-associated adenocarcinoma. *Surgery* 2005; **138**: 924–31.
73 Silberg DG, Furth EE, Taylor JK, Schuck T, Chiou T, Traber PG. CDX1 protein expression in normal, metaplastic, and neoplastic human alimentary tract epithelium. *Gastroenterology* 1997; **113**: 478–86.
74 Mutoh H, Hakamata Y, Sato K *et al*. Conversion of gastric mucosa to intestinal metaplasia in Cdx2-expressing transgenic mice. *Biochem. Biophys. Res. Commun.* 2002; **294**: 470–9.
75 Mutoh H, Sakurai S, Satoh K *et al*. Cdx1 induced intestinal metaplasia in the transgenic mouse stomach: comparative study with Cdx2 transgenic mice. *Gut* 2004; **53**: 1416–23.
76 Silberg DG, Sullivan J, Kang E *et al*. Cdx2 ectopic expression induces gastric intestinal metaplasia in transgenic mice. *Gastroenterology* 2002; **122**: 689–96.
77 Beck F, Chawengsaksophak K, Waring P, Playford RJ, Furness JB. Reprogramming of intestinal differentiation and intercalary regeneration in Cdx2 mutant mice. *Proc. Natl. Acad. Sci USA* 1999; **96**: 7318–23.
78 Litingtung Y, Lei L, Westphal H, Chiang C. Sonic hedgehog is essential to foregut development. *Nat. Genet.* 1998; **20**: 58–61.
79 Madison BB, Braunstein K, Kuizon E, Portman K, Qiao XT, Gumucio DL. Epithelial hedgehog signals pattern the intestinal crypt-villus axis. *Development* 2005; **132**: 279–89.
80 Wang DH, Clemons NJ, Miyashita T *et al*. Aberrant epithelial-mesenchymal Hedgehog signaling characterizes Barrett's metaplasia. *Gastroenterology* 2010; **138**: 1810–22.
81 Dimmler A, Brabletz T, Hlubek F *et al*. Transcription of sonic hedgehog, a potential factor for gastric morphogenesis and gastric mucosa maintenance, is up-regulated in acidic conditions. *Lab. Invest.* 2003; **83**: 1829–37.
82 Lord RV, Salonga D, Danenberg KD *et al*. Telomerase reverse transcriptase expression is increased early in the Barrett's metaplasia, dysplasia, adenocarcinoma sequence. *J. Gastrointest. Surg.* 2000; **4**: 135–42.
83 Brabender J, Marjoram P, Lord RV *et al*. The molecular signature of normal squamous esophageal epithelium identifies the presence of a field effect and can discriminate between patients with Barrett's esophagus and patients with Barrett's-associated adenocarcinoma. *Cancer Epidemiol. Biomarkers Prev.* 2005; **14**: 2113–7.
84 Reid BJ, Li X, Galipeau PC, Vaughan TL. Barrett's oesophagus and oesophageal adenocarcinoma: time for a new synthesis. *Nat. Rev. Cancer* 2010; **10**: 87–101.
85 Galipeau PC, Li X, Blount PL *et al*. NSAIDs modulate CDKN2A, TP53, and DNA content risk for progression to esophageal adenocarcinoma. *PLoS Med.* 2007; **4**: e67.
86 Dunn JM, Mackenzie GD, Oukrif D *et al*. Image cytometry accurately detects DNA ploidy abnormalities and predicts late relapse to high-grade dysplasia and adenocarcinoma in Barrett's

oesophagus following photodynamic therapy. *Br. J. Cancer* 2010; **102**: 1608–17.
87 Fritcher EG, Brankley SM, Kipp BR *et al*. A comparison of conventional cytology, DNA ploidy analysis, and fluorescence in situ hybridization for the detection of dysplasia and adenocarcinoma in patients with Barrett's esophagus. *Hum. Pathol.* 2008; **39**: 1128–35.
88 Jankowski JA, Odze RD. Biomarkers in gastroenterology: between hope and hype comes histopathology. *Am. J. Gastroenterol.* 2009; **104**: 1093–6.
89 Heath EI, Canto MI, Piantadosi S *et al*. Secondary chemoprevention of Barrett's esophagus with celecoxib: results of a randomized trial. *J. Natl. Cancer Inst.* 2007; **99**: 545–57.
90 Das D, Chilton AP, Jankowski JA. Chemoprevention of oesophageal cancer and the AspECT trial. *Recent Results Cancer Res.* 2009; **181**: 161–9.
91 Sikkema M, Kerkhof M, Steyerberg EW *et al*. Aneuploidy and overexpression of Ki67 and p53 as markers for neoplastic progression in Barrett's esophagus: a case-control study. *Am. J. Gastroenterol.* 2009; **104**: 2673–80.
92 Jin Z, Cheng Y, Gu W *et al*. A multicenter, double-blinded validation study of methylation biomarkers for progression prediction in Barrett's esophagus. *Cancer Res.* 2009; **69**: 4112–15.
93 Wang JS, Guo M, Montgomery EA *et al*. DNA promoter hypermethylation of p16 and APC predicts neoplastic progression in Barrett's esophagus. *Am. J. Gastroenterol.* 2009; **104**: 2153–60.
94 Kawakami K, Brabender J, Lord RV *et al*. Hypermethylated APC DNA in plasma and prognosis of patients with esophageal adenocarcinoma. *J. Natl. Cancer Inst.* 2000; **92**: 1805–11.
95 Risques RA, Vaughan TL, Li X *et al*. Leukocyte telomere length predicts cancer risk in Barrett's esophagus. *Cancer Epidemiol. Biomarkers Prev.* 2007; **16**: 2649–55.
96 Wijnhoven BP, Michael MZ, Watson DI. MicroRNAs and cancer. *Br. J. Surg.* 2007; **94**: 23–30.
97 Wijnhoven BP, Hussey DJ, Watson DI, Tsykin A, Smith CM, Michael MZ. MicroRNA profiling of Barrett's oesophagus and oesophageal adenocarcinoma. *Br. J. Surg.* **97**: 853–61.
98 Pouw RE, Gondrie JJ, Rygiel AM *et al*. Properties of the neosquamous epithelium after radiofrequency ablation of Barrett's esophagus containing neoplasia. *Am. J. Gastroenterol.* 2009; **104**: 1366–73.
99 Dijckmeester WA, Wijnhoven BP, Watson DI *et al*. MicroRNA-143 and -205 expression in neosquamous esophageal epithelium following argon plasma ablation of Barrett's esophagus. *J. Gastrointest. Surg.* 2009; **13**: 846–53.
100 Deviere J, Buset M, Dumonceau JM, Rickaert F, Cremer M. Regression of Barrett's epithelium with omeprazole. *N. Engl. J. Med.* 1989; **320**: 1497–8.
101 Brand DL, Ylvisaker JT, Gelfand M, Pope CE, 2nd. Regression of columnar esophageal (Barrett's) epithelium after anti-reflux surgery. *N. Engl. J. Med.* 1980; **302**: 844–8.
102 Bright T, Watson DI, Tam W *et al*. Randomized trial of argon plasma coagulation versus endoscopic surveillance for barrett esophagus after antireflux surgery: late results. *Ann. Surg.* 2007; **246**: 1016–20.
103 Wang KK, Sampliner RE. Updated guidelines 2008 for the diagnosis, surveillance and therapy of Barrett's esophagus. *Am. J. Gastroenterol.* 2008; **103**: 788–97.
104 Abrams JA, Kapel RC, Lindberg GM *et al*. Adherence to biopsy guidelines for Barrett's esophagus surveillance in the community setting in the United States. *Clin. Gastroenterol. Hepatol.* 2009; **7**: 736–42.
105 Bampton PA, Schloithe A, Bull J, Fraser RJ, Padbury RT, Watson DI. Improving surveillance for Barrett's oesophagus. *BMJ* 2006; **332**: 1320–3.
106 Peters FP, Curvers WL, Rosmolen WD *et al*. Surveillance history of endoscopically treated patients with early Barrett's neoplasia: nonadherence to the Seattle biopsy protocol leads to sampling error. *Dis. Esophagus* 2008; **21**: 475–9.
107 Bright T, Schloithe A, Bull JA, Fraser RJ, Bampton P, Watson DI. Outcome of endoscopy surveillance for Barrett's oesophagus. *ANZ J. Surg.* 2009; **79**: 812–16.
108 Barr H, Shepherd NA, Dix A, Roberts DJ, Tan WC, Krasner N. Eradication of high-grade dysplasia in columnar-lined (Barrett's) oesophagus by photodynamic therapy with endogenously generated protoporphyrin IX. *Lancet* 1996; **348**: 584–5.
109 Gondrie JJ, Pouw RE, Sondermeijer CM *et al*. Effective treatment of early Barrett's neoplasia with stepwise circumferential and focal ablation using the HALO system. *Endoscopy* 2008; **40**: 370–9.
110 Overholt BF, Wang KK, Burdick JS *et al*. Five-year efficacy and safety of photodynamic therapy with Photofrin in Barrett's high-grade dysplasia. *Gastrointest. Endosc.* 2007; **66**: 460–8.
111 Ell C, May A, Gossner L *et al*. Endoscopic mucosal resection of early cancer and high-grade dysplasia in Barrett's esophagus. *Gastroenterology* 2000; **118**: 670–7.
112 Pennathur A, Farkas A, Krasinskas AM *et al*. Esophagectomy for T1 esophageal cancer: outcomes in 100 patients and implications for endoscopic therapy. *Ann. Thorac. Surg.* 2009; **87**: 1048–54, discussion 54–5.
113 Overholt BF, Lightdale CJ, Wang KK *et al*. Photodynamic therapy with porfimer sodium for ablation of high-grade dysplasia in Barrett's esophagus: international, partially blinded, randomized phase III trial. *Gastrointest. Endosc.* 2005; **62**: 488–98.
114 Shaheen NJ, Sharma P, Overholt BF *et al*. Radiofrequency ablation in Barrett's esophagus with dysplasia. *N. Engl. J. Med.* 2009; **360**: 2277–88.
115 Ganz RA, Overholt BF, Sharma VK *et al*. Circumferential ablation of Barrett's esophagus that contains high-grade dysplasia: a U.S. multicenter registry. *Gastrointest. Endosc.* 2008; **68**: 35–40.
116 Prasad GA, Wang KK, Halling KC *et al*. Correlation of histology with biomarker status after photodynamic therapy in Barrett esophagus. *Cancer* 2008; **113**: 470–6.
117 Brahmania M, Lam E, Telford J, Enns R. Endoscopic mucosal resection: early experience in British Columbia. *Can. J. Gastroenterol.* 2010; **24**: 239–44.
118 Chennat J, Konda VJ, Ross AS *et al*. Complete Barrett's eradication endoscopic mucosal resection: an effective treatment modality for high-grade dysplasia and intramucosal carcinoma—an American single-center experience. *Am. J. Gastroenterol.* 2009; **104**: 2684–92.
119 Lopes CV, Hela M, Pesenti C *et al*. Circumferential endoscopic resection of Barrett's esophagus with high-grade dysplasia or early adenocarcinoma. *Surg. Endosc.* 2007; **21**: 820–4.