Concepts in the Prevention of Adenocarcinoma of the Distal Esophagus and Proximal Stomach

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ABSTRACT For decades, the incidence rates for squamous cell carcinoma of the esophagus and adenocarcinoma of the distal stomach have been declining while the rates for adenocarcinomas of the esophagus and gastric cardia have increased profoundly. Recent studies have shown that the gastroesophageal junction (GEJ) is regularly exposed to concentrated gastric acid and to a variety of nitrosating species, noxious agents that may contribute to carcinogenesis in this region. For adenocarcinomas that straddle the GEJ, it can be difficult to determine whether the tumor originated in the esophagus or in the gastric cardia. This classification problem hampers studies on the epidemiology and pathogenesis of GEJ tumors. Current concepts in the prevention of cancers of the distal esophagus and proximal stomach have emerged from advances in our understanding of the specific molecular events that occur during the evolution of these tumors. This report reviews those events and focuses on current concepts in the prevention of adenocarcinomas at the GEJ. The similarities and differences in risk factors, molecular pathogenesis, and in preventive strategies for adenocarcinomas of the esophagus and gastric cardia are highlighted. (CA Cancer J Clin 2005;55:334–351.) © American Cancer Society, Inc., 2005.

INTRODUCTION

Cancers of the esophagus and stomach are among the most deadly of all gastrointestinal malignancies, with 5-year mortality rates exceeding 80%.1 For the esophagus, there are two major histological types of cancer – squamous cell carcinoma and adenocarcinoma. Worldwide, more than 90% of all esophageal cancers are squamous cell carcinomas, and this tumor ranks among the world’s 10 most frequent malignancies.2 In a number of Western countries, however, there has been a modest decline in the frequency of squamous cell carcinoma and a dramatic rise in the frequency of adenocarcinoma of the esophagus.3,4 In the United States, for example, the incidence of esophageal adenocarcinoma has increased by approximately 600% over the past few decades, and adenocarcinoma has been the most common histological type of esophageal cancer in this country since the mid1990s.5 The most common histological type of stomach cancer is adenocarcinoma and, worldwide, stomach cancer is the second leading cause of cancer mortality. In Western countries, however, there has been a dramatic decline in the frequency of gastric cancer for more than 50 years.2 This declining frequency has been due to the profound decrease in the incidence of distal gastric cancers. The incidence of cancers of the proximal stomach (the gastric cardia) increased substantially from the 1970s through most of the 1990s, but that incidence may now be declining as well.5

The gastroesophageal junction (GEJ) is the line at which the esophagus ends and the stomach begins. Adenocarcinomas of the GEJ are malignancies that cross the line, so that part of the tumor is in the stomach and part is in the esophagus. There are no clear and universally accepted anatomic landmarks that delimit the distal esophagus and the gastric cardia, and investigations on adenocarcinomas of the GEJ have been hampered by difficulties in ascertaining whether these tumors are esophageal or gastric in origin.6 Most studies on GEJ cancers have categorized the tumors as "cardiac" or esophageal depending on the location of the tumor’s epicenter. Whereas tumors may not grow symmetrically, the location of the tumor epicenter may not accurately identify the origin of a tumor at the GEJ.
A cancer may start in the esophagus and grow distally so that its epicenter eventually is located in the stomach. Consequently, studies that identify GEJ tumors as "cardiac" based on the location of the epicenter will likely include a mixture of both esophageal and gastric cancers. This may explain why the epidemiology of cardiac cancer seems to share features of both esophageal and distal gastric adenocarcinomas (Table 1). This report will focus on current concepts in prevention of adenocarcinomas of the distal esophagus and proximal stomach. Similarities and differences in risk factors, molecular pathogenesis, and in preventive strategies for these cancers will be highlighted.

RISK FACTORS FOR ADENOCARCINOMAS OF THE ESOPHAGUS AND GASTRIC CARDIA

Alcohol and Tobacco Use

Alcohol and tobacco use are strong risk factors for esophageal squamous cell carcinoma in the United States. For adenocarcinomas of the esophagus and gastric cardia, there is only a modest association with tobacco use. In a study conducted by the National Cancer Institute, cigarette smoking was found to increase the risk for adenocarcinoma of the esophagus and gastric cardia by 2.2- and 2.6-fold, respectively. Moreover, the risk of tumor formation associated with cigarette smoking appeared to remain elevated for more than 30 years after the use of tobacco was stopped. Unlike squamous cell carcinoma of the esophagus, for which alcohol abuse is an unequivocal risk factor, the association of alcohol ingestion (even at levels > 70 grams of ethanol per week) with esophageal and gastric adenocarcinoma is disputed and weak at most. Although individuals would be well advised to avoid smoking and heavy use of alcohol, it appears that these prohibitions are unlikely to have a great impact on the frequency of adenocarcinoma of the esophagus and gastric cardia.

Diet and Nutrition

Epidemiological data suggest that the consumption of fruits and vegetables protects against adenocarcinoma of the esophagus and gastric cardia. Despite these data, recent studies suggest a role for dietary nitrate (NO3−), which is found primarily in green leafy vegetables, in promoting carcinogenesis at the GEJ. The nitrate in ingested vegetables is absorbed, concentrated in the salivary glands, and secreted into the mouth where bacteria on the tongue reduce the nitrate to nitrite (NO2−). When the swallowed nitrite first encounters gastric acid, which occurs primarily at the GEJ, a variety of nitrosating species are formed including the potentially toxic gas nitric oxide (NO). High concentrations of NO can be genotoxic and potentially carcinogenic. Recent studies have demonstrated that both the squamous and columnar epithelia surrounding the GEJ experience prolonged exposure to concentrated acid following meals. It is possible that NO, generated when swallowed nitrite meets this acid, facilitates carcinogenesis of the distal esophagus and gastric cardia. It has been proposed that the rising frequency of cancers at the GEJ may be related to the increased nitrate content of vegetables (a consequence of the widespread use of nitrate-based fertilizers after World War II) and the consumption of preserved foods with high nitrate content. It clearly does not seem prudent to discourage salad ingestion as a means to prevent these tumors. To the contrary, most epidemiological data suggest that the consumption of fruits and vegetables protect against these neoplasms.

Obesity

General malnutrition and specific deficiencies of certain vitamins and minerals (including folate, vitamin C, vitamin E, vitamin B6, niacin, and selenium) are strongly associated with squamous cell carcinoma of the esophagus. In contrast, obesity has been established as a strong risk factor for esophageal adenocarcinoma. In the United States, the prevalence of obesity (defined as a body mass index (BMI) ≥ 30) has increased substantially over the past three decades. The risk for developing adenocarcinoma of the esophagus has been shown to increase as BMI increases, with individuals in the upper quartile of BMI having three to seven times the risk as those in
the lowest quartile. In a Swedish study, individuals in the highest quartile for BMI had a 7.6-fold increased risk of esophageal adenocarcinoma and a 2.3-fold increase risk for gastric cardia adenocarcinoma. The mechanism underlying the association between GEJ tumors and obesity is not known but may be related to the fact that obesity may predispose to the development of gastroesophageal reflux disease (GERD).

**GERD**

The major risk factors for esophageal adenocarcinoma are GERD and its sequela Barrett esophagus, the condition in which esophageal squamous epithelium that is damaged by GERD is replaced by a metaplastic, intestinal-type epithelium that is predisposed to malignancy. Indeed, the large majority of esophageal adenocarcinomas are thought to arise from the specialized intestinal metaplasia of Barrett esophagus. In contrast to the unequivocal data linking GERD with Barrett esophagus and esophageal adenocarcinoma, data linking GERD to intestinal metaplasia and cancer of the gastric cardia are contradictory. It has been proposed that cardiac epithelium (a glandular epithelium, comprised almost exclusively of mucus-secreting cells, that can be found in the esophagus as well as in the gastric cardia) may not be a normal lining at all, but rather a metaplastic epithelium that develops as the result of the chronic inflammation induced by exposure of the GEJ to noxious agents like acid, pepsin, and NO. In support of this hypothesis, inflammation of cardiac epithelium (“carditis”) has been found in association with GERD in 47% to 96% of biopsies specimens obtained from the gastric cardia. Chronic carditis may eventuate in intestinal metaplasia which, like the specialized intestinal metaplasia of Barrett esophagus, is predisposed to malignant progression. However, some investigators have found no association between GERD, carditis, and intestinal metaplasia. Data on how the control of GERD may prevent adenocarcinoma at the GEJ are reviewed below.

**Helicobacter pylori**

In 1994, *H. pylori* was classified by the World Health Organization’s International Agency for Research on Cancer as a Group I (definite) carcinogen for adenocarcinoma of the distal stomach because the evidence linking the tumor with this infection was judged to be sufficient. In contrast, *H. pylori* infection has not been identified as a risk factor for esophageal or gastric cardia adenocarcinoma. The virulence of individual strains of *H. pylori* may depend on expression of putative virulence factors such as CagA protein. It appears that infection with *H. pylori*, particularly with the CagA+/Hi1001 strains, actually reduces the risk for these tumors, whereas minimal association has been found between these tumors and CagA–strains. Although *H. pylori* infection clearly causes carditis that would be expected to predispose to cancer development, current data suggest an inverse association between *H. pylori* and the development of gastric cardia adenocarcinoma. The mechanism of this alleged protective effect is not clear, but it has been suggested that *H. pylori* infections that cause a severe pangastritis can decrease gastric acid secretion, thereby protecting against the development of GERD. Thus, GERD may be a stronger risk factor for cardiac cancer than *H. pylori* infection. In any case, eradication of *H. pylori* infection is not likely to decrease the incidence of adenocarcinoma at the GEJ.

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**TABLE 1  Epidemiology of Esophageal and Gastric Adenocarcinoma in the United States**

|                        | Esophagus | Distal Stomach | Gastric Cardia |
|------------------------|-----------|----------------|---------------|
| **Men:Women**          | 7:1       | 2:1            | 4:1           |
| **White:African American** | 4:1       | 1:2            | 2:1           |
| **Association with GERD** | + + +     | 0              | ++            |
| **Association with *H. pylori*** | – –       | +++            | +/-           |
| **Incidence**           | ↑         | ↓              | +/−           |
Adenocarcinomas of the esophagus and gastric cardia are thought to develop through a stepwise process termed the metaplasia-dysplasia-carcinoma sequence (Figure 1). Metaplasia is the process whereby one adult cell type replaces another adult cell type. The replacement of reflux-damaged esophageal squamous epithelium by metaplastic specialized intestinal-type epithelium results in Barrett esophagus. A similar process may occur in the gastric cardia. These metaplastic epithelia are thought to be predisposed to DNA damage that causes dysplasia and frank carcinoma. Underlying these changes in cell phenotype are sequential genetic alterations that bring the normal epithelial cells progressively closer to malignancy. Although adenocarcinomas of the esophagus and cardia share some similar genetic alterations, the exact sequence of molecular changes necessary to produce either adenocarcinoma remains incompletely elucidated. In 2000, Hanahan and Weinberg proposed six essential physiological hallmarks that normal cells must acquire to become cancerous. These cancer hallmarks include the ability to proliferate independent of exogenous mitogenic stimulation, to resist growth-inhibitory signals, to avoid triggering apoptosis, to resist cell senescence, to develop new vascular supplies (angiogenesis), and to invade and metastasize. The sections below will focus on how genetic alterations acquired during the development of adenocarcinomas at the GEJ result in the acquisition of the cancer hallmarks and how this knowledge might be exploited for chemoprevention. Figure 1 summarizes the genetic alterations described below as benign Barrett metaplastic cells progress to esophageal adenocarcinoma.

**Cell Cycle**

Many of the molecular alterations that occur during carcinogenesis in the upper gastrointestinal tract affect genes whose protein products regulate the cell cycle clock apparatus, the key nuclear mechanism which controls whether a cell will proliferate, differentiate, or die. The cell cycle comprises the events that occur in the nucleus between cell divisions. This series of events is divided into four phases: G1 (first gap), S (DNA synthesis), G2 (second gap), and M (mitosis). Near the end of G1, there is a point (termed the restriction or R point) at which the cell “decides” if it will enter S phase and complete the cycle or exit the cycle to enter the resting G0 phase. The retinoblastoma (Rb) protein appears to be the molecular switch that controls the R point. In quiescent cells, hypophosphorylated Rb functions to block progression through the cell cycle. Phosphorylation inactivates Rb, thereby allowing the cell to pass through the R point. The increased rate of proliferation that has been demonstrated in many human tumors reflects an increased proportion of cells passing through the R point.

**Independence from Exogenous Stimulation**

Oncogene expression allows cells to proliferate independent of exogenous mitogenic stimulation. Normal cellular genes that promote cell growth are termed proto-oncogenes. When proto-oncogenes become mutated in such a way that they are overactive, they are called oncogenes. One oncogene implicated in the development of both esophageal and gastric cardia adenocarcinoma is cyclin D1. Cyclin D1 interacts with cyclin-dependent kinases (cdks), which are the enzymes responsible for the inactivation of Rb through phosphorylation. Cyclin D1 overexpression has been found in metaplastic Barrett esophagus, suggesting that it may play an early role in carcinogenesis. Moreover, in Barrett patients who have progressed to cancer, cyclin D1 overexpression has been found in the nondysplastic specialized intestinal-type epithelium more often than in patients who did not develop cancer. In contrast, overexpression of cyclin B1, which mediates the G2 to M transition, has been found in dysplastic Barrett mucosa and esophageal adenocarcinomas but not in metaplastic epithelium alone.
Although cyclin levels are increased in esophageal and gastric cardia adenocarcinomas, it is ultimately the phosphorylation and inactivation of Rb by the cdks that allows cell cycle progression to ensue. Therefore, therapies to prevent cell proliferation have been directed at the cdks. Flavopiridol, a synthetic flavone, is a potent inhibitor of cdk-2 and cdk-4. Despite promising results in a Phase I clinical trial, Phase II trials using flavopiridol as a single agent demonstrated no effect on tumors of the gastric cardia.\textsuperscript{42} Additionally, the study found no effect on tumors of the GEJ, but it is not clear how many of these GEJ tumors arose from the esophagus. Although flavopiridol does not appear to be an effective anticancer agent when used alone, it has shown promise when given in combination with other chemotherapeutic agents. In Phase I clinical trials, patients with esophageal adenocarcinoma treated with flavopiridol in combination with paclitaxel demonstrated either a complete or partial response.\textsuperscript{43} Given these encouraging results, Phase II studies using the combination of flavopiridol and paclitaxel are currently in progress. In addition, preclinical studies in gastric cancer cell lines suggest that flavopiridol administered after treatment with docetaxel may have a potential therapeutic role.\textsuperscript{44} Furthermore, the finding of elevated cyclins in precancerous (metaplastic and dysplastic) Barrett epithelium raises the possibility that agents directed against cdks may have a role as chemopreventive agents.

In addition to the direct activation of oncogenes like the cyclins, tumor cells can proliferate without exogenous stimulation by altering growth factors, growth factor receptors, or the signaling pathways that mediate growth factor-receptor interactions. Activation of growth factor receptors that are members of the tyrosine kinase family can activate the Ras protein and its downstream target, cyclin D1, to promote cellular proliferation.\textsuperscript{45,46} Increased expression of epidermal growth factor (EGF) and transforming growth factor-\(\alpha\) (TGF-\(\alpha\)), have been found in esophageal but not in gastric cardia adenocarcinomas.\textsuperscript{47–49} Increased expression of the EGF receptor (also called ErbB-1) and amplification of 7p12, the chromosomal locus for the EGFR, have been detected in esophageal and gastric cardia adenocarcinomas, respectively.\textsuperscript{47–50} In fact, increased expression of TGF-\(\alpha\) and the EGFR may occur early during esophageal cancer formation, as elevated levels have been found in nondysplastic specialized intestinal metaplasia.\textsuperscript{47,48,51} Whereas the role of the oncogenic form of the normal EGFR family member (erbB-2, also called HER2 or Neu) in the formation of esophageal adenocarcinomas is controversial, data suggest that erbB2 is overexpressed in 20% of gastric cardia tumors.\textsuperscript{37,52–54} Numerous trials have investigated the use of EGFR inhibitors as a potential cancer therapy. The two types of EGFR inhibitors that have been evaluated include receptor blocking monoclonal antibodies and tyrosine ki-
nase inhibitors that inhibit the enzymatic function of the receptor. While numerous clinical trials of these inhibitors have been published, none of the patients in these trials had esophageal or gastric cardia adenocarcinoma.\textsuperscript{55} However, inhibition of EGFR may be a potential therapy for patients with Barrett esophagus and esophageal adenocarcinoma.

Ras proteins (including H-ras and K-ras) and recently the B-raf protein have been identified as important human oncogenes.\textsuperscript{56,57} Ras proteins play central roles in the regulation of normal cell growth. In response to a variety of growth factors and cytokines, Ras proteins initiate a number of downstream signaling pathways that regulate progression through the cell cycle and thus cell proliferation.\textsuperscript{58} Recently, studies have demonstrated K-ras mutations in 11% to 40% of esophageal adenocarcinomas, but few data are available on the frequency of Ras mutations in gastric cardia adenocarcinomas.\textsuperscript{57,59} Moreover, available data do not support an important role for oncogenic B-raf in adenocarcinomas of the upper gastrointestinal tract.\textsuperscript{57,60,61} Given the pivotal role of Ras proteins in the regulation of cell growth, it is not surprising that inhibitors of Ras are in development as anticancer therapies. The enzyme farnesyltransferase (FTase) catalyzes the addition of a farnesyl isoprenoid moiety to Ras, a modification which allows Ras to attach to the inner surface of the plasma membrane and trigger cell signaling.\textsuperscript{58} In a Phase I trial in combination with paclitaxel and carboplatin, FTase inhibitors demonstrated antitumor activity in patients with esophageal adenocarcinoma suggesting a potential therapeutic role for these compounds in the future.\textsuperscript{62} The use of FTase inhibitors for cancer of the gastric cardia has not yet been evaluated.

**Resistance of Growth-Inhibitory Signals**

A number of antigrowth signals converge on Rb to block its phosphorylation, thereby preventing passage through the R point. Normal genes that restrain the cell’s ability to proliferate are termed tumor suppressor genes. Inactivation of tumor suppressor genes in tumor cells occurs by at least three mechanisms, including mutation, deletion of the chromosomal region containing the gene (called loss of heterozygosity (LOH)), or attachment of methyl groups to the promoter region of genes (called promoter methylation), which reduces their expression.

Although Rb is a key tumor suppressor gene, mutation of this gene has not been demonstrated in esophageal adenocarcinomas, and loss of Rb protein expression has been found in only 4% of gastric cardia adenocarcinomas.\textsuperscript{65} It appears that the majority of cancers of the esophagus and gastric cardia inactivate the genes that block Rb function such as p16 and p53. p53 inactivation by LOH of 17p, the p53 locus, and mutation of the remaining allele has been found in approximately 50% to 90% of esophageal adenocarcinomas and in 31% to 63% of gastric cardia adenocarcinomas.\textsuperscript{64–69} Moreover, p53 LOH and mutation have also been found in nonmalignant cells of specialized intestinal metaplasia suggesting that p53 inactivation is an early step in carcinogenesis.\textsuperscript{70–72} The same proliferative effect can be produced by overexpression of an inhibitor of p53, such as MDM2. MDM2 overexpression has been found in 4% of esophageal adenocarcinomas and in 19% of gastric cardia adenocarcinomas.\textsuperscript{67} Of note, MDM2 overexpression has been found only in tumors containing wild-type p53, supporting the notion that increased MDM2 expression is an alternative mechanism for p53 inactivation.\textsuperscript{73} Esophageal and gastric cardia adenocarcinomas also frequently demonstrate allelic loss of 9p21, the chromosomal locus for p16, and methylation of the p16 promoter been reported in 45% to 54% of esophageal adenocarcinomas and in 36% of gastric cardia adenocarcinomas.\textsuperscript{63,69,74–76} Moreover, methylation of p16 has been detected in the nondysplastic, specialized intestinal metaplasia of Barrett esophagus, suggesting that p16 methylation is a very early event in the neoplastic progression of Barrett esophagus. Finally, inactivation of the adenomatous polyposis coli gene (APC) by LOH of 5q21, the APC chromosomal locus, and by promoter methylation has been found in both esophageal and gastric cardia adenocarcinomas as well as in Barrett metaplasia with and without high grade dysplasia.\textsuperscript{68,69,77,78}
The identification of 17p LOH, p53 antibodies, and methylated APC may have a role in predicting neoplastic progression. In a large, prospective trial of patients with Barrett esophagus, 17p LOH in biopsy tissues was found to predict cancer progression at 5 years. Moreover, p53 antibodies have been detected in the sera of Barrett patients who later progressed to dysplasia and cancer. Studies investigating the prognostic significance of 17p LOH and p53 antibodies in gastric cardia tumors have not been done. However, one study found that the risk of cancer of the gastric cardia was threefold higher in patients homozygous for a common genetic polymorphism at codon 72 in the p53 gene that encodes for the amino acid arginine rather than proline. Finally, the detection of methylated APC DNA in the plasma of patients with esophageal adenocarcinoma has been associated with a significantly shortened survival. Although results such as these are promising, large-scale prospective studies are needed to validate the utility of these markers in predicting neoplastic progression in patients with cancers of the distal esophagus and proximal stomach.

Avoidance of Apoptosis

Normal cells have the ability to undergo apoptosis, an innate, cellular self-destruct mechanism. There are a number of ways in which the apoptotic machinery can be activated, including DNA damage, metabolic abnormalities, and death receptor activation. Once activated, the apoptotic machinery converges on an executioner pathway that destroys the cell through a caspase signaling cascade. Apoptosis is beneficial to the organism because it prevents the replication of cells with mutated DNA. However, apoptosis is detrimental to tumor cells, which must find ways to avoid this suicide program if they are to persist.

Inactivation of p53 is one way in which both esophageal and gastric cardia adenocarcinomas avoid triggering apoptosis. Another way is by interfering with the activation of death receptors. For example, the apoptotic machinery can be activated by the binding of the cell surface death receptor Fas with Fas-ligand (FasL). Fas is normally found on lymphocytes and gut epithelial cells, whereas FasL is expressed by activated lymphocytes but not by gut epithelial cells. When bound by FasL, the Fas receptor induces apoptosis in the cell expressing the Fas receptor. Esophageal adenocarcinomas, but not the specialized intestinal metaplasia of Barrett esophagus, have been found to express FasL, an abnormality rendering the tumor cells capable of binding the Fas receptor on the surface of attacking lymphocytes and thereby destroying the immune cells targeted against the tumor. The role of altered death receptor signaling in gastric cardia adenocarcinomas has yet to be investigated extensively. To date, studies investigating the use of antibodies to Fas (which activate the Fas receptor) and the transfer of Fas ligand into tumor-bearing animal models have been disappointing. These novel therapeutic agents have been associated with hepatotoxicity, fulminant hepatic failure, and massive hepatic necrosis.

Finally, cancer cells might avoid apoptosis by increasing the synthesis of an agent that blocks the apoptotic machinery such as cyclooxygenase-2 (COX-2). COX-2 overexpression, which has been shown to reduce the rate of apoptosis in vitro, has been detected in esophageal and gastric cardia adenocarcinomas with expression levels somewhat lower in the latter than in the former. COX-2 overexpression has been detected in biopsies of metaplastic Barrett esophagus, and its expression increases as the cells progress to dysplasia and carcinoma. The role of COX-2 inhibition as a chemopreventive strategy is covered in detail later in this report.

Resistance to Cell Senescence

Senescence refers to the intrinsic mechanisms that limit the proliferative capacity of normal cells. This autonomous mechanism involves shortening of telomeres, which are long stretches of simple, noncoding DNA repeats located on the ends of chromosomes. In normal somatic cells, telomeric DNA is lost with each successive round of cell replication. When telomeres are shortened to a critical length, the
cell is triggered to exit from the cell cycle into a G0 state characterized by permanent growth arrest. Therefore, for cells to become immortal, they must maintain telomere length. Telomerase, the enzyme responsible for the synthesis and maintenance of telomeres, is a protein-RNA complex that uses its RNA as a template for the addition of telomeric sequences to the ends of chromosomes. Most normal somatic cells and tissues lack telomerase, but high levels of telomerase expression have been found in esophageal adenocarcinomas. In contrast, low levels of telomerase have been found in nondysplastic specialized intestinal metaplasia, with a marked increase found during the transition from low-grade to high-grade dysplasia. Few data are available on the expression of telomerase in gastric cardia adenocarcinomas.

Targeting telomerase expression as an anticancer therapy is conceptually appealing because telomerase is present principally in cancer cells. Therefore, inhibitors of telomerase may selectively target cancer cells. The telomerase inhibitor, 2,6-bis[3-[(M-piperidine)propionamido]anthracene-9,10-dione (PPA) has shown promising results as a potential chemotherapeutic agent in in vitro studies of Barrett-associated adenocarcinoma cells. Although there are current Phase I and II clinical trials evaluating telomerase inhibitors, these trials do not involve patients with cancer of the distal esophagus and proximal stomach.

Development of New Vascular Supplies (Angiogenesis)

Essential for tumor growth and metastasis is the formation of new blood vessels, a process termed angiogenesis. Angiogenesis is stimulated by the binding of vascular endothelial growth factors (VEGFs) to their tyrosine kinase receptors (VEGFRs). Following binding, VEGFRs initiate signaling pathways that result in the proliferation and migration of endothelial cells. VEGF mRNA and protein expression have been found to be significantly increased in esophageal adenocarcinomas compared with dysplastic and metaplastic Barrett esophagus and normal esophageal mucosa. No studies to date have specifically investigated the expression of VEGF or VEGFRs in adenocarcinoma of the gastric cardia. VEGF inhibitors have not yet been investigated in adenocarcinomas of the esophagus and gastric cardia. Nevertheless, the recent approval of the first humanized anti-VEGF monoclonal antibody (Avastin, bevacizumab) for the treatment of metastatic colon cancer suggests that this therapeutic approach holds promise.

Invasion and Metastasis

Although the mechanisms are poorly understood, invasion and metastasis of tumor cells are thought to involve abnormalities in cell-cell interaction. Catenins are cytoplasmic proteins attached to the cells' cytoskeleton. The cadherins, a large family of cell adhesion molecules, are anchored by binding to the catenins. Failure of cadherins to interact with catenins can impair cell adhesion and predispose to invasion and metastasis. In addition to its role in cell adhesion, β-catenin is involved in cellular signal transduction by translocating to the nucleus of cells and stimulating the production of genes whose protein products promote cell growth. In normal esophageal squamous mucosa and the nondysplastic, specialized intestinal metaplasia of Barrett esophagus, E-cadherin and β-catenin are found primarily in the cell membrane. As the degree of dysplasia increases, there is a fall in membrane staining for E-cadherin and β-catenin. Moreover, increased cytoplasmic and nuclear staining for these proteins has been found in dysplastic Barrett esophagus. Nuclear accumulation of E-cadherin has been observed in 48% gastric cardia adenocarcinomas.

Matrix metalloproteinases (MMPs) also play an important role in the process of invasion and metastasis. MMPs are a family of zinc-dependent proteolytic enzymes that mediate the destruction of the extracellular matrix. Matrilysin (MMP-7) was found to be the principle MMP in Barrett esophagus and esophageal adenocarcinoma, and its presence correlated with aggressiveness of the tumor. A genetic polymorphism in MMP-2 (CC genotype) has been associated with a three-fold increase in the incidence of gastric cardia adenocarcinoma.

The importance of
MMPs in tumor invasion and metastasis has led to the search for clinically useful inhibitors as anticancer agents. Thus far pseudopeptides that mimic MMP substrates, nonpeptide molecules that bind zinc which is needed for MMP function, and AE-941, an extract from shark cartilage with an unknown MMP inhibitory function, have been investigated. Unfortunately, the clinical trials have been unsuccessful, making the future of MMP inhibitor therapy for adenocarcinomas of the esophagus and gastric cardia unclear.

CANCER PREVENTION STRATEGIES

GERD and Barrett esophagus are the major risk factors for adenocarcinoma at the GEJ. Patients with Barrett esophagus develop adenocarcinoma at the rate of approximately 0.5% per year, and the rate of cancer development for patients who have GERD without Barrett esophagus is substantially lower than that. When assessing potential cancer preventive strategies for adenocarcinomas at the GEJ, physicians should consider the implications of this low absolute risk of cancer development. One method that can be used to assess the clinical utility of a treatment is to calculate the number needed to treat (NNT), using the formula $NNT = \frac{1}{ARR}$ (where $ARR$ is the absolute risk reduction achieved by the treatment).

No treatment, medical or surgical, has been proved to decrease the risk of adenocarcinoma in Barrett esophagus (see below). For the sake of argument, assume that there is a highly effective treatment that will reduce that risk by one-half, ie, from 0.50% to 0.25% per year. This represents an absolute risk reduction of 0.25%. In this example, therefore, the $NNT = \frac{1}{0.0025} = 400$. Thus, even if there were a highly effective cancer-preventive treatment for Barrett esophagus, 400 patients would need to be treated to prevent one cancer in 1 year. Such a large NNT can be acceptable only if the treatment is reasonably inexpensive, convenient, and safe.

Endoscopic Screening and Surveillance for Barrett Esophagus

To decrease mortality from adenocarcinoma at the GEJ, it has been proposed that patients with GERD symptoms should be screened endoscopically for Barrett esophagus, and that patients found to have Barrett esophagus should have regular endoscopic surveillance to look for dysplasia (a potentially curable form of neoplasia). The rationale for this proposal includes the following assumptions, all of which are unproved and highly questionable: 1) Screening will reliably identify those individuals at highest risk for developing esophageal adenocarcinoma, 2) Without intervention, patients with Barrett esophagus will have decreased survival because of deaths from adenocarcinoma, 3) Surveillance will reliably detect dysplasia in Barrett esophagus, and 4) Treatment of the dysplasia found by surveillance will prolong survival and improve quality of life by preventing death and morbidity from esophageal cancer.

It is not clear that screening patients with GERD symptoms reliably identifies those individuals at high risk for esophageal adenocarcinoma, and studies suggest that 40% of patients with esophageal adenocarcinoma have no history of such symptoms. Consequently, screening programs that target only patients with heartburn can have only limited impact on cancer mortality rates, and there is little evidence that these programs have prevented deaths from esophageal adenocarcinomas. In published series of patients found to have these tumors, fewer than 5% were known to have had Barrett esophagus before they sought medical attention for symptoms of esophageal cancer.

A number of studies have suggested that survival for patients with Barrett esophagus does not differ significantly from that for age- and sex-matched control subjects in the general population. However, those studies were comprised of predominantly older patients who often succumbed to common comorbid illnesses such as coronary artery disease rather than to esophageal adenocarcinoma. Proponents of surveillance argue that the results of those studies may not be applicable to younger, healthier patients with Barrett esophagus.
Observational studies have documented that endoscopic surveillance can detect curable neoplasms in Barrett esophagus, and that asymptomatic cancers discovered during surveillance are less advanced than those found in patients who present with cancer symptoms like dysphagia and weight loss.\textsuperscript{116–119} Those studies are highly susceptible to a number of biases, however (eg, healthy volunteer bias, lead-time bias, length-time bias), and it is not appropriate to conclude that surveillance is beneficial based on the observation that patients with asymptomatic neoplasms survive longer than patients who have symptoms due to esophageal cancer.\textsuperscript{109} No study has established the reliability of surveillance in detecting curable dysplasia, and a number of reports have documented the development of incurable malignancies in some patients despite adherence to endoscopic surveillance programs.\textsuperscript{116,117} Furthermore, hazardous invasive therapies for dysplasia like esophagectomy ultimately might do more harm than good.

Some computer models have suggested that endoscopic screening and surveillance for Barrett esophagus can be beneficial provided that certain baseline assumptions are valid.\textsuperscript{120–123} One group used a decision tree to explore the utility of one-time endoscopic screening for high-grade dysplasia in 60-year-old patients with GERD symptoms. The model estimated that screening might cost $24,700 per life-year saved, provided that a number of baseline conditions were favorable (eg, relatively high prevalence of dysplasia in the group screened, low cost for endoscopy, good health-related quality of life after esophagectomy).\textsuperscript{121} Another group used a Markov model to construct a computer cohort simulation of 10,000 middle-aged patients with Barrett esophagus and assumed that esophagectomy would be performed for those whose surveillance endoscopies showed high-grade dysplasia. For an annual cancer incidence rate of 0.4\%, this analysis suggested that endoscopic surveillance performed every 5 years was the preferred strategy, costing $98,000 per quality-adjusted life-year gained.\textsuperscript{120} Another, more recent cost-utility analysis contradicted the findings of the latter model, concluding that whereas screening for Barrett esophagus might be cost-effective, surveillance is not.\textsuperscript{123} None of these computer models can be considered definitive, however, because all incorporate numerous layers of soft data and questionable assumptions.

In a recent editorial, Spechler summarized the dilemma regarding screening and surveillance for Barrett esophagus as follows: 1) Endoscopy is expensive, 2) There is no proof that endoscopic screening of patients with GERD for Barrett esophagus has any impact on survival, 3) No “proof” in the form of a randomized controlled trial is likely to become available in the near future, 4) Available observational studies, which are subject to numerous forms of bias, suggest that screening and surveillance are beneficial, 5) Available computer models, which are based on some soft data and questionable assumptions, suggest that screening can be beneficial, 6) Although endoscopic screening clearly can be associated with risks (ie, complications resulting both from endoscopy and from the invasive procedures used to treat conditions found by endoscopy), no study has shown an overall survival disadvantage for patients in screening and surveillance programs.\textsuperscript{124} In this murky situation, where the indirect evidence available suggests that screening and surveillance are beneficial and the major objection is cost, it may be better to err by performing unnecessary endoscopy rather than by missing curable esophageal neoplasms. A surveillance strategy recommended by the Practice Parameters Committee of the American College of Gastroenterology is outlined in Figure 2.\textsuperscript{125} It is important to recognize that this strategy was formulated based primarily on expert opinions, and that studies have not demonstrated the efficacy of this approach.

### Chemoprevention

**Control of Acid Reflux**

### Prevention of Barrett Esophagus

It is not clear why only a minority of patients with GERD develop Barrett esophagus, but recent studies from our laboratory suggest that the esophageal squamous epithelium of those...
patients may be predisposed to healing through metaplasia rather than through the regeneration of more squamous cells. We investigated the response of the extracellular regulated kinase (ERK)1/2, an enzyme involved in stimulating cell proliferation, following acid exposure of the squamous esophagus of GERD patients with and without Barrett esophagus. We found that baseline levels of ERK1/2 were significantly lower in the squamous mucosa of the GERD patients without Barrett esophagus. Moreover, we found that acid exposure increased the activity of ERK1/2 in the squamous epithelium of GERD patients without Barrett esophagus but not in patients with Barrett esophagus. It is possible that individuals who have high baseline levels of ERK1/2 and who fail to activate this proliferative pathway in response to acid exposure may be predisposed to healing through intestinal metaplasia rather than through squamous regeneration, but further studies are necessary to prove this contention. Nevertheless, it seems likely that early treatment of patients with GERD might prevent this first step in the metaplasia-dysplasia-carcinoma sequence. Our studies further suggest that it may someday be possible to identify a molecular biomarker that would identify GERD patients predisposed to develop Barrett esophagus. Such a biomarker might enable the selection of a subgroup of GERD patients who might benefit from aggressive acid suppressive therapy and screening.

**Prevention of Cancer in Patients with Barrett Esophagus**

The aggressive control of gastric acid secretion has been proposed as a strategy to decrease the risk of cancer in Barrett esophagus. This proposal is based primarily on data from clinical studies suggesting that acid suppression decreases proliferation in Barrett esophagus. In one study, cell proliferation and differentiation were studied in biopsy specimens of Barrett esophagus before and after 6 months of therapy with proton pump inhibitors (PPIs). Cellular proliferation decreased and differentiation increased in the pa-

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**Figure 2  Endoscopic surveillance strategy modified slightly from the recommendations of the Practice Parameters Committee of the American College of Gastroenterology.** (Note that the recommendation for endoscopic mucosal resection and ablative therapies in patients with high-grade dysplasia who are not fit for surgery or for whom a protocol is available is not recommended specifically by the ACG.)
tients who exhibited normalization of esophageal acid exposure by 24-hour ambulatory pH monitoring but not in those with persistently abnormal acid exposure despite PPI treatment. A more recent study showed that Barrett patients treated with PPIs developed dysplasia less frequently than those treated with histamine H2-receptor antagonists, which are less effective at controlling gastric acid secretion. Furthermore, a significantly increased rate of cell proliferation and proproliferative cell cycle abnormalities have been detected in biopsies of Barrett epithelium from patients treated with H2-receptor antagonists compared with biopsies from patients treated with PPIs. However, this approach of using aggressive acid suppression (ie, more than that required to eliminate symptoms and heal esophagitis) is not without potential drawbacks. For example, chronic PPI therapy leads to elevations in serum gastrin levels, which have been linked to increased proliferation in Barrett biopsy specimens in vitro. Overall, the clinical data support the approach of potent acid suppression as a chemopreventive strategy in patients with Barrett esophagus. However, controlled, prospective clinical trials are still needed before this can be recommended for widespread practice. Presently, we feel that patients with Barrett esophagus should be treated with PPIs given in a dose that eliminates the symptoms and endoscopic signs of GERD. At this time, we do not advocate routine 24-hour esophageal pH monitoring to document the normalization of esophageal acid exposure with PPIs.

Surgeons have proposed that antireflux surgery (fundoplication) might be more effective than antisecretory therapy for preventing cancer in Barrett esophagus. This proposal is based on weak evidence, however. For example, two small, uncontrolled studies found fewer cases of dysplasia and cancer among patients with Barrett esophagus who had antireflux surgery than among those who had received medical treatment. Some even have proposed that antisecretory therapy might predispose to malignancy, and that the increasing use of antisecretory medications might underlie the rising frequency of esophageal adenocarcinoma in Western countries. However, the limited studies that have addressed this issue directly have not found a significant association between esophageal adenocarcinoma and the use of antisecretory agents per se.

A report describing the long-term outcome of a randomized trial of medical and surgical therapies for 247 veteran patients with complicated GERD (including 108 with Barrett esophagus) does not support the contention that fundoplication prevents esophageal cancer better than medical antireflux therapy. During 10 to 13 years of follow-up, 4 of 165 patients (2.4%) in the medical group and 1 of 82 (1.2%) in the surgical group developed an esophageal adenocarcinoma. The difference between the treatment groups in the incidence of this tumor was not statistically significant but, with such a low observed rate of cancer development, the study did not have sufficient statistical power to detect small differences in the incidence of esophageal cancer. However, any potential cancer-preventive benefit of surgery was offset by an unexplained, but significant, decrease in survival for the surgical patients due to excess deaths from heart disease. Another report describing the results of a large, Swedish, population-based cohort study also refutes the contention that antireflux surgery prevents esophageal adenocarcinoma. In this study, patients with GERD were followed for up to 32 years. The relative risk for developing esophageal adenocarcinoma (compared with the general population) among 35,274 men who received medical antireflux therapy was 6.3 (95% CI 4.5-8.7), whereas the relative risk for 6,406 men treated with fundoplication was 14.1 (95% CI 8.0-22.8). A recent meta-analysis also found no significant cancer-protective effect of antireflux surgery.

We feel that antireflux surgery should not be advised with the expectation that the procedure will prolong life by preventing esophageal cancer.

Inhibition of Ornithine Decarboxylase

Ornithine decarboxylase (ODC) is the rate-limiting enzyme in the synthesis of polyamines that are essential for cells to progress through the cell cycle. Elevated levels of
polyamines and ODC activity have been found in colonic polyps and colon cancer as well as in the min mouse model of familial adenomatous polyposis coli.\textsuperscript{145–148} Treatment of min mice with α-difluoromethylornithine (DFMO), a reversible inhibitor of ODC, has been shown to suppress tumorigenesis, suggesting that ODC may be a target for chemopreventive therapies.\textsuperscript{148} Inhibitors of ODC have also been found to decrease tumor formation in animal models of bladder, breast, and skin carcinogenesis.\textsuperscript{149–151} DFMO also has been used in clinical trials as a chemopreventive and chemotherapeutic agent, but its clinical utility has been limited by its side effect of ototoxicity.\textsuperscript{152–156}

Barrett specialized intestinal metaplasia exhibits greater expression of ODC than normal squamous or gastric epithelia.\textsuperscript{157,158} Moreover, expression of ODC has been found to increase significantly as dysplastic changes develop in the metaplastic mucosa.\textsuperscript{157,159} In vitro, DFMO has been shown to reduce growth of metaplastic Barrett epithelial cells.\textsuperscript{157} In clinical trials, treatment of patients with Barrett esophagus for 6 weeks with low dose DFMO (0.5 g/m\textsuperscript{2}) resulted in an approximately 60% decrease in polyamine content in metaplastic Barrett mucosa as well as in normal control epithelia.\textsuperscript{160} At least one Barrett patient treated with DFMO has developed irreversible ototoxicity, however, and it is not clear that lowering the polyamine content in Barrett esophagus will alter the cancer risk.\textsuperscript{161}

Other, indirect inhibitors of ODC may have a potential role for chemoprevention in Barrett esophagus. For example, troglitazone, a peroxisome proliferator-activated receptor gamma (PPAR-gamma) ligand, has been found to reduce ODC activity in human esophageal adenocarcinoma cells.\textsuperscript{162} In vitro treatment of a human esophageal adenocarcinoma cell line with troglitazone significantly inhibited cell growth and induced apoptosis, events which would limit the growth of neoplastic cells in vivo.\textsuperscript{162} Moreover, the in vitro effect of troglitazone was specific for esophageal adenocarcinoma cells as there was no effect of troglitazone on cell growth, apoptosis, or ODC activity in an esophageal squamous cell carcinoma cell line.\textsuperscript{162} Taken together, these data suggest that ODC inhibition may be a promising chemopreventive strategy in patients with Barrett esophagus, but well designed, controlled clinical trials are needed before any of the available ODC inhibitors can be recommended for clinical use.

\textbf{Inhibition of COX-2}

Inhibitors of cyclo-oxygenase (COX) have received considerable attention as potential chemopreventive agents in Barrett esophagus. COXs mediate the production of prostaglandins from arachidonic acid. Two isoforms have been identified: COX-1 and COX-2. COX-1 is expressed constitutively, whereas COX-2 expression can be induced by cytokines, growth factors, and tumor promoters.\textsuperscript{163} Increased expression of COX-2, but not COX-1, has been detected in a number of human gastrointestinal tumors.\textsuperscript{164–166} In vitro, enhanced expression of COX-2 has been shown to promote proliferation and decrease apoptosis.\textsuperscript{167} Inhibition of COX-2 by nonsteroidal anti-inflammatory drugs (NSAIDs) has been shown to reverse these effects, suggesting that these agents might be used as chemopreventive agents.\textsuperscript{167,168}

A number of epidemiologic studies suggest that the use of aspirin and other NSAIDs may protect against the formation of gastrointestinal tumors, including adenocarcinoma of the esophagus, but such a protective effect has not been established for adenocarcinoma of the gastric cardia.\textsuperscript{169–172} Although numerous studies suggest that the antitumor effect of NSAIDs is effected by inhibition of COX-2 rather than COX-1, there are also data to suggest that some of these antitumor effects are independent of COX inhibition.\textsuperscript{173,174} Regardless of the precise mechanism, NSAIDs have been shown to be an effective chemopreventive strategy for certain gastrointestinal tumors.

Selective inhibitors of COX-2 have been used in both in vivo and in vitro studies of Barrett esophagus. The COX-2 selective agents have been shown to decrease proliferation and increase apoptosis in vitro in combined primary cultures of dysplastic and nondysplastic Barrett epithelial cells and in human Barrett-associated esophageal adenocarcinoma cell lines.\textsuperscript{175,176} In an animal model of Barrett esophagus, selective inhibition of COX-2 has been shown to decrease both the
development of Barrett esophagus and the incidence of esophageal adenocarcinoma.\textsuperscript{177,178} Moreover, selective inhibition of COX-2 has been shown to decrease proliferation in metaplastic Barrett mucosa in vivo.\textsuperscript{179}

Nonselective NSAIDs may also have chemopreventive effects. In Barrett-adenocarcinoma cells in vitro and in an animal model of Barrett esophagus, the administration of nonselective NSAIDs induced apoptosis and decreased the risk of tumor formation.\textsuperscript{177,180} Moreover, there was no significant difference in the risk of tumor formation in animals treated with MF-tricyclic (a selective COX-2 inhibitor) and Sulindac (a nonselective NSAID).\textsuperscript{177} Taken together, these results suggest that NSAIDs may be useful in altering the rate of neoplastic progression in patients with metaplastic Barrett esophagus.

The risk of cardiovascular side effects limits the utility of the COX-2 selective agents for chemoprevention in Barrett esophagus. Aspirin and other nonselective NSAIDs may have cardioprotective effects, but these agents can have serious gastrointestinal side effects. A number of patients with Barrett esophagus have cardiovascular risk factors, and aspirin may be both cardioprotective and chemopreventive in that population. There is an ongoing trial (AspECT) in the United Kingdom investigating the effect of aspirin combined with PPI therapy on neoplastic progression in patients with Barrett esophagus.\textsuperscript{181,182} The results of this study are eagerly awaited. Presently, it is not clear that the potential chemopreventive effects of NSAIDs outweigh their toxicities.

**CONCLUSION**

Over the past 2 decades, the frequency of adenocarcinoma at the GEJ has increased profoundly. The major risk factors for esophageal adenocarcinoma are GERD and its sequela, Barrett esophagus. The role of GERD in the genesis of gastric cardia adenocarcinomas remains controversial. Despite advances in endoscopic technology, screening and surveillance strategies for early detection of these tumors have had limited efficacy in preventing these deadly tumors. Therefore, it has become increasingly important to understand the pathogenesis of esophageal and gastric cardia adenocarcinomas at the molecular level to develop more targeted cancer-preventive strategies. The clinical utility of the experimental data discussed above on selectively targeting acid exposure, ODC, and COX-2 remains questionable and the results of well-designed, prospective clinical studies to establish the efficacy of these approaches are eagerly awaited (Table 2).

**REFERENCES**

1. El Rafai W, Powell SM. Molecular biology of gastric cancer. Semin Radiat Oncol 2002;12:128–140.
2. Crew KD, Neugut AI. Epidemiology of upper gastrointestinal malignancies. Semin Oncol 2004;31:450–464.
3. Devesa SS, Blot WJ, Fraumeni JF, Jr. Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. Cancer 1998;83:2049–2053.
4. Devesa SS, Fraumeni JF, Jr. The rising incidence of gastric cancer. J Natl Cancer Inst 1999;91:747–749.
5. Poli H, Welch HG. The role of overdiagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence. J Natl Cancer Inst 2005;97:142–146.
6. Spechler SJ. The role of gastric cardiac in metaplasia and neoplasia at the gastroesophageal junction. Gastroenterology 1999;117:218–228.
7. Brown LM, Silverman DT, Potters LM, et al. Adenocarcinoma of the esophagus and esophagogastric junction in white men in the United States: alcohol, tobacco, and socioeconomic factors. Cancer Causes Control 1994;5:333–340.
8. Gammon MD, Schoenberg JB, Ahn H, et al. Tobacco, alcohol, and socioeconomic status and adenocarcinomas of the esophagus and gastric cardia. J Natl Cancer Inst 1997;89:1277–1284.
9. Lagergren J, Bergstrom R, Lindgren A, Nyren O. The role of tobacco, snuff and alcohol use in the etiology of cancer of the oesophagus and gastric cardia. Int J Cancer 2000;85:340–346.
10. Kabat GC, Ng SK, Wynder EL. Tobacco, alcohol intake, and diet in relation to adenocarcinoma of the esophagus and gastric cardia. Cancer Causes Control 1993;4:123–132.
11. Brown LM, Swanson CA, Gridley G, et al. Adenocarcinoma of the esophagus: role of obesity and diet. J Natl Cancer Inst 1995;87:104–109.
12. Iijima K, Henry E, Muria A, et al. Dietary nitrate generates potentially mutagenic concentrations of nitric oxide at the gastroesophageal junction. Gastroenterology 2002;122:1248–1257.
13. Bartholomew B, Hill MJ. The pharmacology of dietary nitrate and the origin of urinary nitrate. Food Chem Toxicol 1984;22:789–795.
14. Duncan C, Dougall H, Johnston P, et al. Chemical generation of nitric oxide in the mouth from the enterosalivary circulation of dietary nitrate. Nat Med 1995;1:546–551.
15. McKnight GM, Smith LM, Drummond RS, et al. Chemical synthesis of nitric oxide in the stomach from dietary nitrate in humans. Gut 1997;40:211–214.
16. Spechler SJ. Carcinogenesis at the gastroesophageal junction: free radicals at the frontier. Gastroenterology 2002;122:1518–1520.
17. Fletcher J, Wiz A, Young J, et al. Unbuffered highly acidic gastric juice exists at the gastroesophageal junction after a meal. Gastroenterology 2001;121:775–783.
18. Mayne ST, Risch HA, Dubrow R, et al. Nutrient intake and risk of subtypes of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev 2001;10:1055–1062.
19. Mark SD, Qiao YL, Dawson SM, et al. Prospective study of serum selenium levels and incident esophageal and gastric cancers. J Natl Cancer Inst 2000;92:1753–1763.
20. Franceschi S, Bidoli E, Negri E, et al. Role of macronutrients, vitamins and minerals in the aetiology of squamous-cell carcinoma of the oesophagus. Int J Cancer 2000;86:626–631.
21. Brown LM, Devesa SS. Epidemiologic trends in esophageal and gastric cancer in the United States. Surg Oncol Clin N Am 2002;11:235–256.
22. Lagerrjen G, Bergstrom R, Nyren O. Association between body mass and adenocarcinoma of the esophagus and gastric cardia. Ann Intern Med 1999;130:883–890.
23. Lagerrjen G, Bergstrom R, Lindgren A, Nyren O. Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma [see comments]. N Engl J Med 1999;340:825–831.
24. Spechler SJ. Clinical practice. Barrett’s Esophagus. N Engl J Med 2002;346:836–842.
25. Oberg S, Peters JH, DeMeester TR, et al. Inflammation and specialized intestinal metaplasia of cardiac mucosa is a manifestation of gastroesophageal reflux disease. Ann Surg 1997;226:522–530.
26. Ciendes A, Smok G, Burdiles P, et al. “Carditis”: an objective histological marker for pathologic gastroesophageal reflux disease. Dis Esophagus 1998;11:101–105.
27. Goldblum JR, Vicari JJ, Falk GW, et al. Inflammation and intestinal metaplasia of the gastric cardia: the role of gastroesophageal reflux and H. pylori infection. Gastroenterology 1998;114:633–639.
28. Chen YY, Antonioli DA, Spechler SJ, et al. Gastroesophageal reflux disease versus Helicobacter pylori infection as the cause of gastric carditis. Mod Pathol 1998;11:950–956.
29. Ye W, Held M, Lagerrjen J, et al. Helicobacter pylori infection and gastric atrophy: risk of adenocarcinoma and squamous-cell carcinoma of the esophagus and adenocarcinoma of the gastric cardia. J Natl Cancer Inst 2004;96:388–396.
30. Spechler SJ, Fischbach L, Feldman M. Clinical aspects of genetic variability in Helicobacter pylori. JAMA 2000;283:1264–1266.
31. Choy WH, Blaser MJ, Blot WJ, et al. An inverse relation between cagA+ strains of Helicobacter pylori infection and risk of esophageal and gastric cardia adenocarcinoma. Cancer Res 1998;58:588–590.
32. Hansen S, Melby KK, Aase S, et al. Helicobacter pylori infection and risk of cancer of the stomach and non-cardia gastric cancer. A nested case-control study. Scand J Gastroenterol 1999;34:353–360.
33. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57–70.
34. Pardee AB. A restriction point for control of normal animal cell proliferation. Proc Natl Acad Sci U S A 1974;71:1286–1290.
35. Weinberg RA. The retinoblastoma protein and cell cycle control. Cell 1995;81:323–330.
36. Arber N, Gammon MD, Hibshoosh H, et al. Overexpression of cyclin D1 occurs in both squamous carcinomas and adenocarcinomas of the esophagus and in adenocarcinomas of the stomach. Hum Pathol 1999;30:1087–1092.
37. Wens MM, Kupers EJ, Hemmes MA, et al. Barrett’s adenocarcinomas resemble adenocarcinomas of the gastric cardia in terms of chromosomal copy number changes, but relate to squamous cell carcinomas of the distal oesophagus with respect to the presence of high-level amplifications. J Pathol 2003;199:157–165.
38. Arber N, Lightdale C, Rotterdam H, et al. Increased expression of the cyclin D1 gene in Barrett’s esophagus. Cancer Epidemiol Biomarkers Prev 1996;5:457–459.
39. Bani-Hani K, Martin IG, Hardie LJ, et al. Prospective study of cyclin D1 overexpression in Barrett’s esophagus: association with increased risk of adenocarcinoma. J Natl Cancer Inst 2000;92:1316–1321.
40. Sarbia M, Bektas N, Muller W, et al. Expression of cyclin E in dysplasia, carcinoma, and non-malignant lesions of Barrett’s esophagus. Cancer Epidemiol Biomarkers Prev 1999;8:2597–2601.
41. Geddert H, Heep HJ, Gabbert HE, Sarbia M. Expression of cyclin B1 in the metaplasia-dysplasia-carcinoma sequence of Barrett esophagus. Cancer 2002;94:212–218.
42. Schwartz GK, Ilson D, Saltz L, et al. Phase II study of the cyclin-dependent kinase inhibitor flavopiridol administered to patients with advanced gastric carcinoma. J Clin Oncol 2001;19:1985–1992.
43. Schwartz GK, O’Reilly E, Ilson D, et al. Phase I study of the cyclin-dependent kinase inhibitor flavopiridol in combination with paclitaxel in patients with advanced solid tumors. J Clin Oncol 2002;20:2157–2170.
44. Motwani M, Rizzo C, Sirotnak F, et al. Flavopiridol enhances the effect of docetaxel in vitro and in vivo in human gastric cancer cells. Mol Cancer Ther 2003;2:549–555.
45. Lundberg AS, Weinberg RA. Control of the cell cycle and apoptosis. Eur J Cancer 1999;35:531–539.
46. Liu JJ, Chao JR, Jiang MC, et al. Ras transformation results in an elevated level of cyclin D1 and acceleration of G1 progression in NIH 3T3 cells. Mol Cell Biol 1995;15:3654–3663.
47. Jankowski J, Hopwood D, Wormley KG. Flow-cytometric analysis of growth-regulatory peptides and their receptors in Barrett’s oesophagus and oesophageal adenocarcinoma. Scand J Gastroenterol 1992;27:147–154.
48. Bro MJ, Filippe MI, Linehan J, Jankowski J. Association of transforming growth factor alpha (TGFα) and its precursors with malignant change in Barrett’s epithelium: biological and clinical variants. Int J Cancer 1998;70:27–32.
49. Yacoub L, Goldmann H, Odze RD. Transforming growth factor-alpha, epithelial growth factor receptor, and MiB-1 expression in Barrett’s-associated neoplasia: correlation with prognosis. Mod Pathol 1997;10:105–112.
50. van Dekken H, Geelen E, Djinns WN, et al. Comparative genomic hybridization of cancer of the gastroesophageal junction: deletion of 14q31-32.1 discriminates between esophageal (Barrett’s) and gastric cardia adenocarcinomas. Cancer Res 1999;59:748–752.
51. Jankowski J, McMenemy R, Hopwood D, et al. Abnormal expression of growth regulatory factors in Barrett’s oesophagus. Clin Sci (Colch) 1991;81:663–668.
52. Jankowski J, Coghill G, Hopwood D, Wormley KG. Oncogenes and onco-suppressor gene in adenocarcinoma of the oesophagus. Gut 1992;33:1033–1038.
53. Flejou JF, Paraf F, Muzzeau F, et al. Expression of c-erbB-2 oncoprotein in Barrett’s adenocarcinoma: pathological and prognostic correlations. J Clin Pathol 1994;47:23–26.
54. Brien TP, Odze RD, Sheehan CE, et al. HER-2/neu gene amplification by FISH predicts poor survival in Barrett’s esophagus-associated adenocarcinoma. Hum Pathol 2000;31:33–39.
55. El Rayes BF, LoRusso PM. Targeting the epidermal growth factor receptor. Br J Cancer 2004;91:418–424.
56. Cooper GM. Cellular transforming genes. Science 1982;217:801–806.
57. Sommerer F, Vieth M, Markworth A, et al. Mutations of BRAF and KRAS2 in the development of Barrett’s adenocarcinoma. Oncogene 2004;23:554–558.
58. Rowinsky EK, Windle JJ, Von Hoff DD. RAs protein farnesyltransferase: A strategic target for anticancer therapeutic development. J Clin Oncol 1999;17:3631–3652.
59. Lord RV, O’Grady R, Sheehan C, et al. K-ras codon 12 mutations in Barrett’s oesophagus and adenocarcinomas of the oesophagus and oesophagogastric junction. J Gastroenterol Hepatol 2000;15:730–736.
60. Trautmann B, Wittekind C, Strobel D, et al. K-ras point mutations are rare events in premalignant forms of Barrett’s oesophagus. Eur J Gastroenterol Hepatol 1996;8:779–804.
61. Melter S, Manz SM, Wood PK, et al. Activation of c-Ki-ras in human gastrointestinal dys-
plasms determined by direct sequencing of polymerase chain reaction products. Cancer Res 1990;50:3627–3630.

62. Dy GK, Brzezuk LM, Croghan GA, et al. A phase I trial of the novel farnesyl protein transferase inhibitor, BMS-214662, in combination with paclitaxel and carboplatin in patients with advanced cancer. Clin Cancer Res 2005;11:1877–1883.

63. Kim MA, Lee HS, Yang HK, Kim WH. Clinicopathologic and protein expression differences between cardia carcinoma and noncardia carcinoma of the stomach. Cancer 2005;103:1439–1446.

64. Hamelin R, Flejou JF, Muzeau F, et al. TP53 gene mutations and p53 protein immunoreactivity in malignant and premalignant Barrett’s esophagus. Gastroenterology 1994;107:1012–1018.

65. Galipeau PC, Prevo LJ, Sanchez CA, et al. Clonal expansion and loss of heterozygosity at chromosomes 9p and 17p in premalignant esophageal (Barrett’s) tissue. J Natl Cancer Inst 1999;91:2087–2095.

66. Meltzer SJ, Yin J, Huang Y, et al. Reduction to homozygosity involving p53 in esophageal cancers demonstrated by the polymerase chain reaction. Proc Nad Acad Sci USA 1991;88:4976–4980.

67. Taniere P, Martel-Planché G, Maurici D, et al. Molecular and clinical differences between adenocarcinomas of the esophagus and of the gastric cardia. Am J Pathol 2001;158:33–40.

68. Yanagi M, Keller G, Mueller J, et al. Comparison of loss of heterozygosity and microsatellite instability in adenocarcinomas of the distal esophagus and proximal stomach. Virchows Arch 2000;437:605–610.

69. Marsman WA, Birjinhooh RN, Van Rees BP, et al. Loss of heterozygosity and immuno-histochemistry of adenocarcinomas of the esophagus and gastric cardia. Clin Cancer Res 2004;10:8479–8485.

70. Blount PL, Ramel S, Raskind WH, et al. Allelic deletions at p53 gene overexpression in Barrett’s adenocarcinoma. Cancer Res 1991;51:5482–5486.

71. Barrett MT, Sanchez CA, Prevo LJ, et al. Evolution of neoplastic cell lineages in Barrett esophagus. Nat Genet 1999;22:106–109.

72. Cason AG, Mukhopadhyay T, Cleary KR, et al. p53 gene mutations in Barrett’s epithelium and esophageal cancer. Cancer Res 1991;51:4495–4499.

73. Lin J, Beermann DG. Molecular biology of upper gastrointestinal malignancies. Semin Oncol 2004;31:476–486.

74. Barrett MT, Sanchez CA, Galipeau PC, et al. Allelic loss of 9p21 and mutation of the CDKN2/ p16 gene develop as early lesions during neoplastic progression in Barrett’s esophagus. Oncogene 1996;13:1867–1873.

75. Wong DJ, Barrett MT, Stoger R, et al. p16INK4a promoter is hypermethylated at a high frequency in esophageal adenocarcinomas. Cancer Res 1997;57:2619–2622.

76. Sarbia M, Geddert H, Klump B, et al. Hypermethylation of tumor suppressor genes (p16INK4a, p14ARF and APC) in adenocarcinomas of the upper gastrointestinal tract. Int J Cancer 2004;111:224–228.

77. Dolan K, Garde J, Walker SJ, et al. LOH at the sites of the DCC, APC, and TP53 tumor suppressor genes occurs in Barrett’s metaplasia and dysplasia adjacent to adenocarcinoma of the esophagus. Hum Pathol 1999;30:1508–1514.

78. Eads CA, Lord RV, Kurumoo SK, et al. Fields of aberrant CpG island hypermethylation in Barrett’s esophagus and associated adenocarcinoma. Cancer Res 2000;60:5021–5026.

79. Reid BJ, Prevo LJ, Galipeau PC, et al. Rab99inovich PS. Predictors of progression in Barrett’s esophagus II. baseline 17p (p53) loss of heterozygosity identifies a patient subset at increased risk for neoplastic progression. Am J Gastroenterol 2001;96:2839–2848.

80. Crawley HM, Meltzer SJ, De Benedetti VM, et al. Anti-p53 antibodies in patients with Barrett’s esophagus or esophageal carcinoma can predict cancer diagnosis. Gastroenterology 1998;115:19–27.

81. Zhang ZW, Newcomb P, Hollowood A, et al. Age-associated increase of codon 72 Arginine p53 frequency in gastric cardia and non-cardia adenocarcinoma. Clin Cancer Res 2003;9:2151–2156.

82. Kawakami K, Crabender J, Lord RV, et al. Hypermethylated APC DNA in Plasma and Prognosis of Patients With Esophageal Adenocarcinoma. J Natl Cancer Inst 2000;92:1805–1811.

83. Ehts SW. To die or not to die: an overview of apoptosis and its role in disease. JAMA 1998;279:300–307.

84. Suda T, Takahashi T, Golstein P, Nagata S. Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. Cell 1993;75:1169–1178.

85. Younges M, Schwartz MR, Ertan A, et al. Fas ligand expression in esophageal carcinomas and their lymph node metastases. Cancer 2000;88:524–528.

86. Lacronique V, Mignon A, Fabre M, et al. Bcl-2 protects from lethal hepatic apoptosis induced by an anti-Fas antibody in mice. Nat Med 1996;2:80–86.

87. Aoki K, Akyurek LM, San H, et al. Re-expression of cyclooxygenase-2 (COX-2) in Barrett’s adenocarcinoma sequence: correlation with disease progression and dedifferentiation. Proc Natl Acad Sci USA 1991;88:4976–4980.

88. Elder DJ, Halton DE, Hague A, Paraskeva C. Fas and Fas ligand expression in gastroesophageal reflux disease, Barrett’s esophagus, and esophageal adenocarcinoma: an immunohistochemical and immunomblot study. Am J Gastroenterol 1995;90:1808–1813.

89. Washington K, Chiappori A, Hamilton K, et al. Expression of beta-catenin, alpha-catenin, and E-cadherin in Barrett’s esophagus and esophageal adenocarcinomas. Mod Pathol 1998;11:805–813.

90. Aberle H, Schwartz H, Kemler R. Cadherin-catenin complex: protein interactions and their implications for cadherin function. J Cell Biochem 1996;61:514–523.

91. Swami S, Kumbale S, Triadafilopoulos G. E-cadherin expression in gastroesophageal reflux disease, Barrett’s esophagus, and esophageal adenocarcinoma: an immunohistochemical and immunoblot study. Am J Gastroenterol 1995;90:1808–1813.

92. Bailey T, Biddlestone I, Shepherd N, et al. Altered cadherin and catenin complexes in the Barrett’s esophagus-dysplasia-adenocarcinoma sequence: correlation with disease progression and dedifferentiation. Am J Pathol 1998;152:135–144.

93. Seery JP, Syrigos KN, Karayiannakis AJ, et al. Abnormal expression of the E-cadherin-catenin complex in dysplastic Barrett’s oesophagus. Acta Oncol 1999;38:945–948.

94. Coussens LM, Fingleton B, Matrisian LM. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science 2002;295:2387–2392.
1-methyl-1-nitrosourea. Carcinogenesis 1986;7:837–840.
151. Verma AK. Inhibition of tumor promotion by DL-alpha-difluoromethylornithine, a specific, irreversible inhibitor of ornithine decarboxylase. Basic Life Sci 1990;52:195–204.
152. Schweitzer VG. Ototoxicity of chemotherapy agents. Otolaryngol Clin North Am 1993;26:759–789.
153. Love RR, Carbone PP, Verma AK, et al. Randomized phase I chemoprevention dose-seeking study of alpha-difluoromethylornithine. J Natl Cancer Inst 1993;85:732–737.
154. Meyskens FL, Jr., Gerner EW, Emerson S, et al. Effect of alpha-difluoromethylornithine on rectal mucosal levels of polyamines in a randomized, double-blinded trial for colon cancer prevention. J Natl Cancer Inst 1998;90:1212–1218.
155. Meyskens FL, Jr., Gerner EW. Development of difluoromethylornithine (DFMO) as a chemoprevention agent. Clin Cancer Res 1999;5:948–951.
156. Mitchell MF, Tortolero-Luna G, Lee JJ, et al. Phase I dose de-escalation trial of alpha-difluoromethylornithine in patients with grade 3 cervical intraepithelial neoplasia. Clin Cancer Res 1998;4:303–310.
157. Garewal HS, Gerner EW, Sampliner RE, Roe D. Ornithine decarboxylase and polyamine levels in columnar upper gastrointestinal mucosa in patients with Barrett’s esophagus. Cancer Res 1988;48:3288–3291.
158. Gray MR, Wallace HM, Goulding H, et al. Mucosal polyamine metabolism in the columnar lined oesophagus. Gut 1993;34:584–587.
159. Garewal HS, Sampliner R, Gerner E, et al. Ornithine decarboxylase activity in Barrett’s esophagus: a potential marker for dysplasia. Gastroenterology 1988;94:819–821.
160. Garewal HS, Sampliner RE, Fennerty MB. Chemopreventive studies in Barrett’s esophagus: a model premalignant lesion for esophageal adenocarcinoma. J Natl Cancer Inst Monogr 1992;51:54.
161. Lao CD, Backoff P, Shotland LI, et al. Irreversible ototoxicity associated with difluoromethylornithine. Cancer Epidemiol Biomarkers Prev 2004;13:1250–1252.
162. Takashima T, Fujiwara Y, Higuchi K, et al. PPAR-gamma ligands inhibit growth of human esophageal adenocarcinoma cells through induction of apoptosis, cell cycle arrest and reduction of ornithine decarboxylase activity. Int J Oncol 2001;19:465–471.
163. Simmons DL, Levy DB, Yannoni Y, Erikson RL. Identification of a phorbol ester-repressible v-src-inducible gene. Proc Natl Acad Sci U S A 1989;86:1178–1182.
164. Eberhart CE, Coffey RJ, Radhika A, et al. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology 1994;107:1183–1188.
165. Tucker ON, Dannenberg AJ, Yang EK, et al. Cyclooxygenase-2 expression is up-regulated in human pancreatic cancer. Cancer Res 1999;59:987–990.
166. Steinbach G, Lynch PM, Phillips RK, et al. The effect of Celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med 2000;342:1946–1952.
167. Ding XZ, Tong WG, Adrian TE. Blockade of cyclooxygenase 2 inhibitors proliferation and induces apoptosis in human pancreatic cancer cells. Anticancer Res 2000;20:2625–2631.
168. Kawamori T, Rao CV, Seibert K, Reddy BS. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, against colon carcinogenesis. Cancer Res 1998;58:409–412.
169. Giardello FM, Hamilton SR, Krush AJ, et al. Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. N Engl J Med 1993;328:1313–1316.
170. Thun MJ, Namboodiri MM, Calle EE, et al. Aspirin use and risk of fatal cancer [see comments]. Cancer Res 1993;53:1322–1327.
171. Farrow DC, Vaughan TL, Hansten PD, et al. Use of aspirin and other nonsteroidal anti-inflammatory drugs and risk of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev 1998;7:97–102.
172. Greenberg ER, Baron JA, Freeman DHJ, et al. Reduced risk of large-bowel adenomas among aspirin users. The Polyp Prevention Study Group. J Natl Cancer Inst 1993;85:912–916.
173. Molina MA, Sitja-Armu M, Lemoine MG, et al. Increased cyclooxygenase-2 expression in human pancreatic carcinomas and cell lines: growth inhibition by nonsteroidal anti-inflammatory drugs. Cancer Res 1999;59:4356–4362.
174. Piazza GA, Rahm AL, Krutzsch M, et al. Antineoplastic drugs sulindac sulfide and sulfone inhibit cell growth by inducing apoptosis. Cancer Res 1995;55:3110–3116.
175. Souza RF, Shewmake K, Beer D.G., et al. Selective inhibition of cyclooxygenase-2 suppresses growth and induces apoptosis in human esophageal adenocarcinoma cells. Cancer Res 2000;60:5767–5772.
176. Buttar NS, Wang KK, Anderson MA, et al. The effect of selective cyclooxygenase-2 inhibition in Barrett’s oesophagus epithelium: an in vitro study. J Natl Cancer Inst 2002;94:422–429.
177. Buttar NS, Wang KK, Leontovich O, et al. Chemoprevention of esophageal adenocarcinoma by COX-2 inhibitors in an animal model of Barrett’s oesophagus. Gastroenterology 2002;121:1101–1112.
178. Oyama K, Fujimura T, Ninomuya I, et al. A COX-2 inhibitor prevents the esophageal inflammation-metaplasia-adenocarcinoma sequence in rats. Carcinogenesis 2005;26:565–570.
179. Kaur BS, Khanmeehi N, Ivani M, et al. Rofecoxib inhibits cyclooxygenase 2 expression and activity and reduces cell proliferation in Barrett’s oesophagus. Gastroenterology 2002;123:60–67.
180. Aggarwal S, Taneja N, Lin L, et al. Indomethacin-induced apoptosis in esophageal adenocarcinoma cells involves upregulation of Bax and translocation of mitochondrial cytochrome C independent of COX-2 expression [In Process Citation]. Neoplasia 2000;2:346–356.
181. Raj A, Jankowski J. Acid suppression and chemoprevention in Barrett’s oesophagus. Dig Dis 2004;22:171–180.
182. Jankowski J, Sharma P. Review article: approaches to Barrett’s oesophagus treatment—the role of proton pump inhibitors and other interventions. Aliment Pharmacol Ther 2004;19 Suppl 1:S4–59.