

Review

Molecular Evolution of the Metaplasia-Dysplasia-Adenocarcinoma Sequence in the Esophagus

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The incidence of adenocarcinoma of the esophagus has been increasing in developing countries over the last three decades and probably reflects a genuine increase in the incidence of its recognized precursor lesion, Barrett's metaplasia. Despite advances in multimodality therapy, the prognosis for invasive esophageal adenocarcinoma is poor. An improved understanding of the molecular biology of this disease may allow improved diagnosis, therapy, and prognosis. We focus on recent developments in the molecular and cell biology of Barrett's metaplasia, a heterogeneous lesion affecting the transitional zone of the gastro-esophageal junction whose associated molecular alterations may vary both in nature and temporally. Early premalignant clones produce biological and genetic heterogeneity as seen by multiple p53 mutations, p16 mutations, aneuploidy, and abnormal methylation resulting in stepwise changes in differentiation, proliferation, and apoptosis, allowing disease progression under selective pressure. Abnormalities in expression of growth factors of the epidermal growth factor family and cell adhesion molecules, especially cadherin/catenin complexes, may occur early in invasion. Exploitation of these molecular events may lead to a more appropriate diagnosis and understanding of these lesions in the future. (*Am J Pathol* 1999, 154:965-973)

Gastroesophageal reflux disease is arguably the most common medical condition in Western countries; 30% of adults complain of heartburn at least once per month.¹ Chronic esophagitis has been shown to limit physical and social activity, resulting in quality of life scores as poor as those provided by angina patients awaiting coronary bypass surgery. Forty percent of patients with esophagitis will improve spontaneously, 50% will have persistent esophagitis, and up to 10% will progress to Barrett's esophagus (BE).²⁻⁴ Evidence indicates that the prevalence of BE³ and its sequelae are both increasing, especially in the sixth decade of life in males.² There is compelling etiological evidence that acid refluxate is the major factor in progression from benign esophagitis to BE.¹ The association between pathological acid exposure and esophagitis, especially in short segment Barrett's metaplasia, is, however, only 60%, which suggests that in up to 40% of cases other factors like nocturnal bile,⁴ nonsteroidal anti-inflammatory drugs, radiotherapy, chemotherapy,¹ caustic agents, nitrosamines, *Helicobacter* colonization, or familial predisposition may be causative.⁵

The classic endoscopic feature of BE is the presence of salmon pink mucosa. Histologically, the presence of specialized intestinal metaplasia containing goblet cells is characteristic (Figure 1). Short-segment Barrett's esophagus (SSBE), ie, Barrett's metaplasia less than 3 cm in length, is found in 8-20% of adult individuals, making it more prevalent than long-segment Barrett's esophagus (LSBE) (1% adult prevalence)⁶⁻¹⁰ (Table 1). Despite this fact, only 35% of esophageal adenocarcinomas arise in SSBE; therefore, the true cancer risk in SSBE

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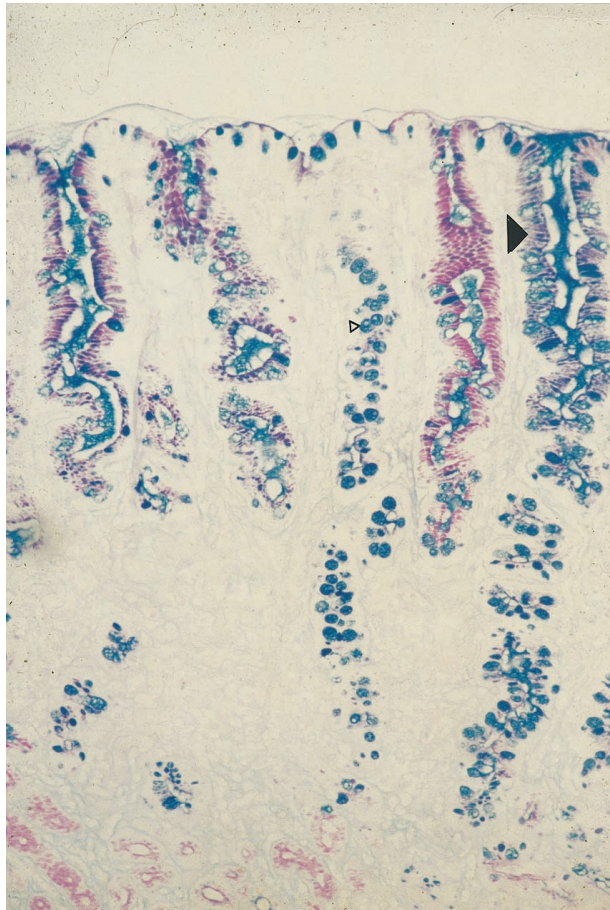


Figure 1. Photomicrograph of intestinal metaplasia in Barrett's esophagus stained with Alcian blue/periodic acid-Schiff (mucins). Barrett's esophagus is composed of columnar lined mucous-secreting cells and a proportion of the glands will be composed of goblet cells (small arrowhead). Alcian blue diastase periodic acid-Schiff staining indicates the heterogeneity of mucin phenotypes in esophageal cells: blue (basic), red (neutral), and purple (mixed) mucins (large arrowhead). Original magnification, $\times 250$.

is presently unclear but probably lies between 0.03–1%¹⁰ (Table 1). SSBE and, less commonly, LSBE have also been reported in at least one study to be associated with the occurrence of esophago-gastric adenocarcinomas and specialized intestinal metaplasia of the gastric car-

dia.¹⁰ Esophageal and gastric cardia adenocarcinomas also share many features including increasing incidence,^{11,12} male gender bias, tumor histology,¹³ and common antigens such as bile duct mucins and large intestinal antigens.¹⁴ Conversely, the risk factors, incidence, histopathology, and molecular biology of esophageal adenocarcinoma differ dramatically from those of squamous cell carcinoma. In particular, squamous cell carcinoma is associated with a poor diet,¹⁵ cigarette smoking, and low socioeconomic status,¹⁶ whereas adenocarcinoma is associated with obesity¹⁷ and white race and is more prevalent in Caucasians in the richer North American and European countries.¹⁸

To date no treatments have been shown to reverse the progression of Barrett's esophagus completely and convincingly or to alter its natural history once it has developed.¹⁹ Moreover, developed photodynamic therapy has recently been associated with the subsequent occurrence of unusual neoplastic lesions lying deep in the submucosa.¹⁹ Even after prolonged high-dose proton pump inhibition or successful antireflux surgery, fewer than 10% of Barrett's cases regress and progression to cancer may occur over a short span of 3 years.^{3,20} Cancers detected in endoscopic surveillance programs have a better prognosis, characterized by 5-year survival rates of 35–45% compared with 5–15% rates in cancers occurring outside surveillance populations, even when allowing for lead bias and earlier staging of detected lesions.^{21,22}

Genetic and Epigenetic Events Leading to Loss of Genomic Stability

Although the colorectal adenoma-carcinoma sequence (ACS) model has become the paradigm for researchers in molecular oncology,²³ a similar mechanistic representation is only now becoming accepted for the development of Barrett's adenocarcinoma. Barrett's is a heterogeneous metaplasia in which 2–5% of cases will have a lifetime risk of Barrett's adenocarcinoma, the metaplasia-dysplasia-adenocarcinoma sequence (MCS).³ MCS differs from ACS in several important regards. First, Barrett's

Table 1. Comparison of Long Segment BE, Short Segment BE, and Specialized Intestinal Metaplasia (SIM) at the Esophago-gastric Junction

| | Long segment BE | Short segment BE | SIM at the gastro-esophageal junction | Reference |
|---------------------|-----------------|------------------|---------------------------------------|-----------|
| Prevalence | 1.3% | 8–17% | 18–36% | 6, 7 |
| GERD | +++ | ++ | – | 6 |
| <i>Helicobacter</i> | + | ++ | +++ | 6, 7 |
| Racial bias | Caucasian | Caucasian | None | 18 |
| Age bias | +++ | +++ | +++ | 6, 18 |
| Gender bias | Male | Male | – | 8, 9, 10 |
| Esophageal cancer | ++ | + | +/- | 6–10 |
| Gastric cancer | +/- | + | – | 10 |
| Migration* | +++ | + | – | * |
| Glandular dysplasia | ++ | + | +/- | 10 |

SIMEGJ is very common but has little association with gastro-esophageal reflux disease malignant potential.

GERD, gastro-esophageal reflux disease; +++, very common (>50% of cases); ++, moderately common (>10% of cases); +, not common (<5% of cases); –, very uncommon (<1% of cases).

*Expanding dye fronts in Barrett's tissue after 0.5 ml intraepithelial tattooing with India ink (J. Jankowski, MD thesis, Dundee University, Dundee, UK).

metaplasia, even when dysplastic, is rarely polypoid like colorectal adenoma. This has been attributed by some researchers to the high frequency of Ki-ras and adenomatous polyposis coli gene (APC) mutations in the colon and rectum, whereas these alterations are very uncommon in Barrett's dysplasia. However, it seems that these latter genes, while permitting polypoid growth, may not be sufficient on their own, as many tumors expressing them are nonpolypoid. Second, colorectal adenomas arise in *de novo* epithelium, whereas in Barrett's esophagus premalignant lesions arise in metaplastic tissue containing goblet cells.²⁴ Third, Barrett's metaplasia arises in a background of reflux-induced chronic inflammation and ulceration,²⁵ whereas this does not occur in the ACS. In this regard, Barrett's neoplastic progression does bear some similarity to that seen in idiopathic inflammatory bowel disease.

The progression of Barrett's metaplasia to adenocarcinoma is associated with several changes in gene structure, gene expression, and protein structure.²⁶⁻³⁴ The following sequence of events is not conclusive and is presented merely to reflect the potential interplay of multiple molecular pathways in the progression to adenocarcinoma. Perhaps one of the earliest molecular events is the selection and propagation of the metaplastic clones with specialized intestinal metaplasia (Figure 2). Subsequently, loss of cell cycle check points and genomic instability may contribute to slow clonal expansion perhaps by increasing proliferation^{32,33} (Figure 3). Inhibition of apoptosis in BE occurs late, and then only in a select proportion of cells with high grade dysplasia. Invasive cancer may be preceded by alteration of cell adhesion,³⁴ whereas subsequent cumulative genetic errors may result in the generation of multiple clones of transformed cells, thereby expanding the population of altered cells with an angiogenic or metastatic potential.

Pathophysiology of Chronic Esophagitis: Restitution and Replication

The development of esophagitis represents the failure of many mucosal defenses of the esophagus to counteract the refluxed acid or gastroduodenal contents.³⁵ The buffering activity of alkaline saliva and esophageal mucus and the esophageal peristaltic clearance are, we believe, usually sufficient to prevent mucosal damage from infrequent, transient lower esophageal sphincter relaxations. If, however, reflux is frequent or prolonged, episodes of gastro-esophageal reflux occur and tissue damage results, initially affecting the cells of the superficial compartment (Figure 2). The regenerating inflamed epithelium contains immature squamous cells that are sensitive to acid or bile damage.³⁶⁻³⁹

The proliferative hierarchy of the normal squamous cell-lined esophagus is relatively well understood. However, the mechanisms whereby focal areas of native squamous mucosa are replaced by metaplastic tissue are less certain; we present one favored current hypothesis. One of the early adaptive responses to increased cell loss in reflux esophagitis is an increase of the proliferative zone height to maintain or increase epithelial thickness by trophic stimulation of locally produced epidermal growth factor⁴⁰ (Figure 2A). In addition, there is also an increased proliferative zone length as a result of folding of the basal epithelium (papillae formation).³ The functional stem cells in the basal zone at the tip of the papillae remain in a relatively superficial position³⁰ in the epithelium, making them far more accessible and susceptible to refluxed or ingested chemical mutagens permeating through the thin upper layers than their counterparts in the flat basal layer deeper in the mucosa (Figure 2B). Mucosal repair occurs more rapidly when reflux disease is treated, especially with the combined actions of epithelial migration and connective tissue contraction.³⁴ In 10% of cases, when treatment is insufficient the mucosal breach is more quickly and effectively replaced by *de novo* Barrett's metaplasia (Figure 2C).

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Formation of Metaplasia: Replacement by Metaplastic Epithelium

Although the origin of BE is a matter of conjecture, one current theory holds that the stem cells of squamous mucosa or associated glandular ducts undergo altered differentiation, producing both microvilli and intercellular ridges, and express unique glandular phenotypes distinct from adjacent mucosal gastric stem cells.⁴¹⁻⁴⁴ This Barrett's metaplastic lineage may give rise to Paneth cells and neuroendocrine cells in addition to gastric and intestinal cells and is therefore pluripotent.⁴² Current theory indicates that these cells give rise to intestinal-type metaplasia. However, skeptics argue that gastric-type and fundic-type metaplasias are also discernible and that the three metaplastic types may more accurately be referred to as a mosaic, although a convincing paradigm is lacking (Figure 1). The reason behind this heterogeneity of metaplastic phenotypes is unclear but the proportion of each has been attributed in part to the composition of the refluxate (environment).⁴⁰ The appearance of metaplasia during esophageal regeneration may also theoretically be selected for by several factors, including the degree of local stem cell enrichment, clonality,³⁴ number of DNA adducts accrued and alterations of xenobiotic metabolizing enzymes (mutagenesis),⁴⁵ and expression of homeobox genes such as members of the *cdx* family (differentiation pathways) (P. Traber, personal correspondence). Phenotypic heterogeneity may also be controlled genetically because clonal divergence in chromosomes 5, 8, 9, 12, 17, and 18 in nondysplastic Barrett's cells can also be identified.^{46,47}

The location and composition of the proliferative compartment in the crypts of the metaplastic epithelium are not as well defined as in columnar lined epithelia of the stomach.⁴² Interestingly, the degree to which differentiation occurs varies considerably. BE that appears in childhood differs from the adult variety in that intestinal mucins and cytokeratins are not present.⁴⁸ The adult variety also has an inflammatory cell infiltrate and may have *Helicobacter*-like organisms, both of which are less common in juvenile metaplasia.⁴⁹ Barrett's intestinal phenotype has

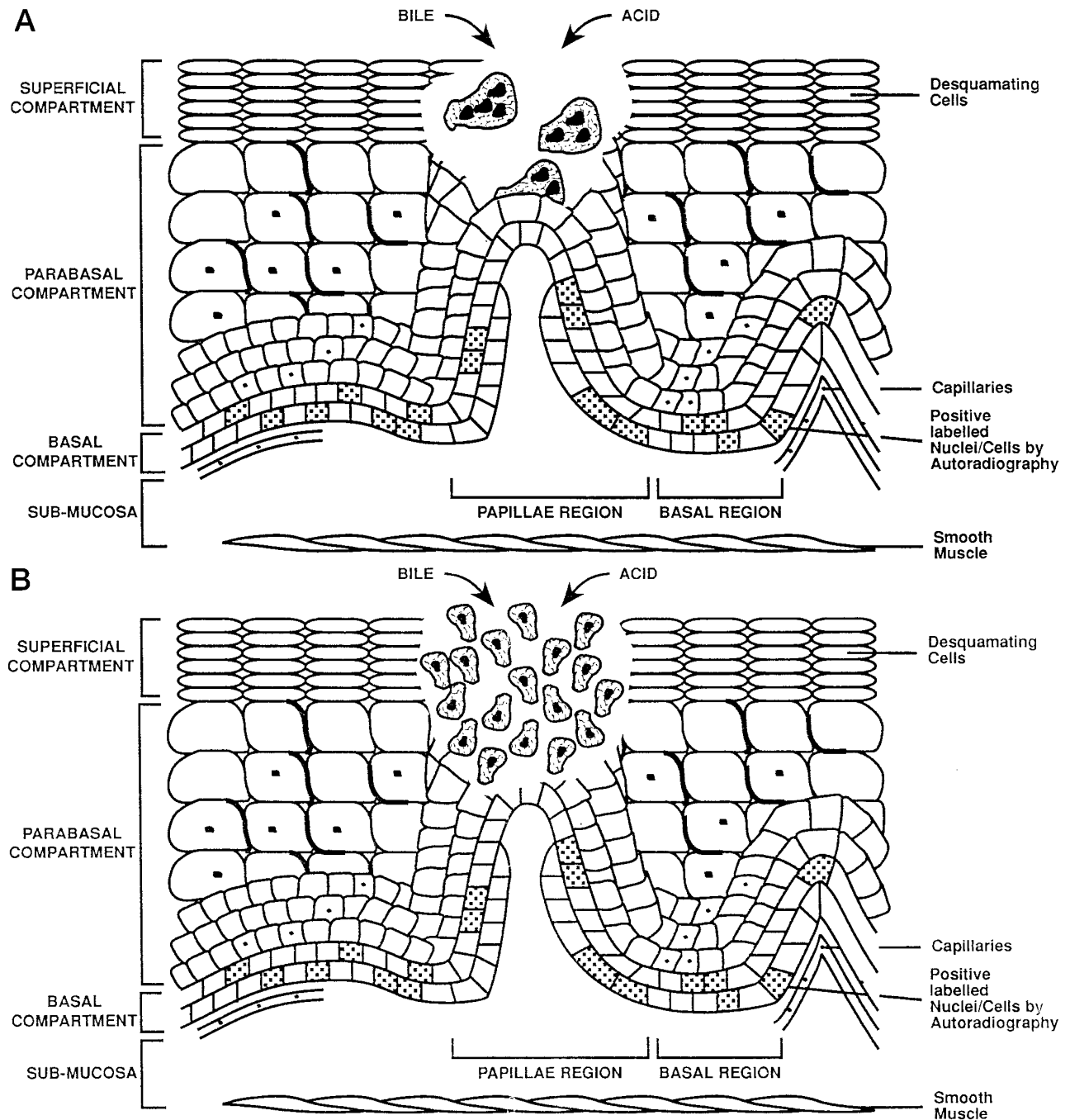


Figure 2. (Legend on next page)

higher proliferative indices; this is associated with altered expression of multiple growth factors and inducible nitric oxide synthase (iNOS; NOS-2) and cyclooxygenase-2.^{21,50}

The replacement by LSBE has been reported in one study to be very rapid, with the maximal proximal colonization of the esophagus occurring within 3 years of initiation. Furthermore, once formed, the rate of surface area remains constant in most individuals. Only 5–10% of cases progress in surface area and 0–2% may partially regress in surface area.^{10,35} More data are required

about the natural history of benign Barrett's metaplasia before these observations can be confirmed.

Dysplasia and Aneuploidy: Clonal Expansion by Increased Cell Cycle Abnormalities and Migration

Although the true prevalence of high grade dysplasia and aneuploidy are unknown because of referral bias, they have been reported in 2–24% of individuals with Barrett's

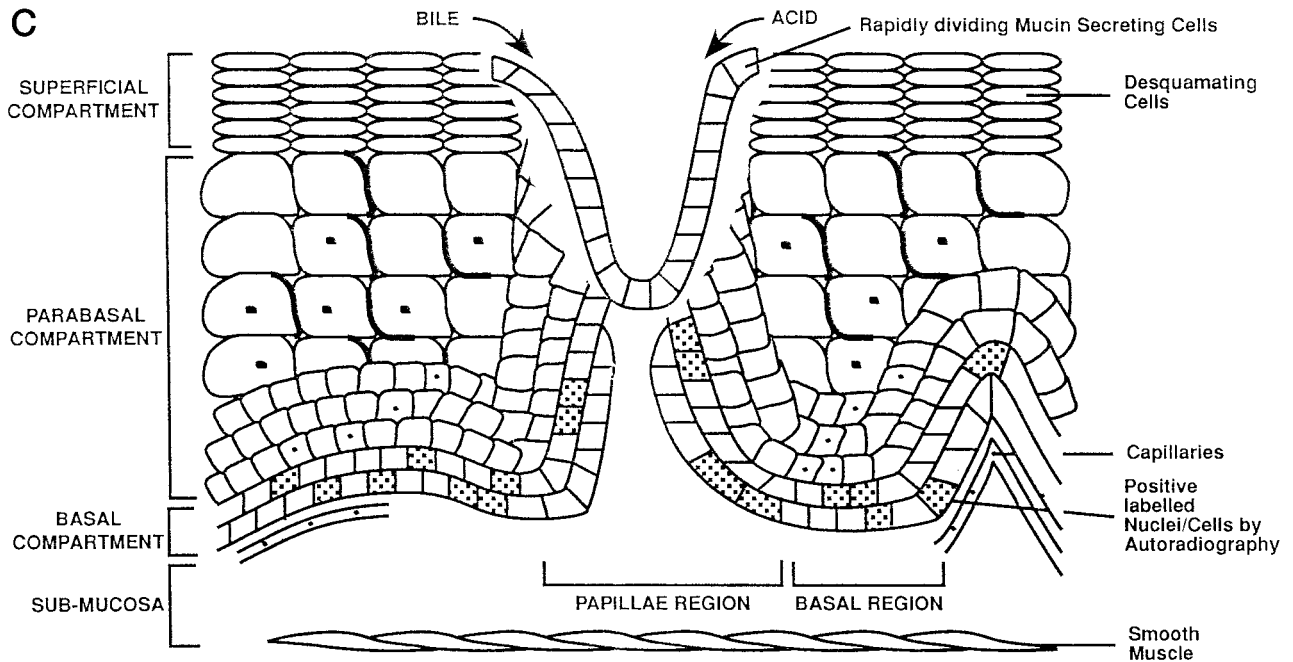


Figure 2. Schematic representation of adaptation during Barrett's mucosa formation. The three compartments of the esophageal epithelium are represented on the diagrams. The bottom of each diagram shows basal compartment containing both stem cells (speckled nucleus) and proliferating cells; the middle, parabasal compartment containing proliferating cells; and the top, superficial compartment containing only mature differentiated cells. The uninflamed mucosa (right) is flat, whereas the inflamed mucosa (left) has invaginations of the basal layer, termed papillae. **A:** Damage to the esophageal differentiated cells in the superficial and parabasal compartments of the esophagus. **B:** Damage to a deeper compartment involving the squamous epithelial stem cells in the basal compartment of the papillae. **C:** Generation of clones with a mucin-secreting lineage resistant to acid/bile.

metaplasia and have a fourfold to eightfold greater risk of developing cancer¹⁰ compared with the more common low grade dysplasia.⁵¹⁻⁵³ These high grade dysplastic lesions may already have irreversibly progressed; at least 50% have immediately adjacent adenocarcinoma and a variable proportion of the rest may remain static for at least 1 to 3 years regardless of the presence or absence of refluxed gastric or duodenal contents.^{53,54}

Dysplastic cells may have proliferative controls that are relaxed or uncoupled from the appropriate regulatory cues. In part this may be a result of altered expression of cytokines and growth factors,⁴⁰ although the acquisition of genomic alterations of cell cycle-associated genes also occurs. These cell cycle genes include increased cyclin D1 expression (chromosome 11q13),⁵⁵ hypermethylated or mutated p16 (chromosome 9p21), and mobilization of cells from G₀ to G₁ with subsequent accumulation in the G₂ phase³³ (Figure 3). Identification of increased telomerase RNA in early dysplastic lesions including Barrett's metaplasia has been reported.⁵⁶ p53 mutations occur in only 1-5% of metaplastic diploid cell populations but are present in most aneuploid cells, suggesting they are usually not early events.^{29,57-59} Furthermore, p53 is mutated increasingly in exons 5-8 during MCS: in 5-10% of cases with indeterminate dysplasia, in 65% of those with low grade dysplasia, in 75% of cases with high grade dysplasia, and in 50-90% of esophageal adenocarcinomas, suggesting that p53 mutations occur more often later in progression.⁶⁰⁻⁶² Epigenetic alterations in the expression of growth factors and their receptors, especially of the epidermal growth factor family, are also associated with these cell cycle changes in

dysplastic Barrett's mucosa. In particular, we believe increased expression of TGF α and its precursor, prepro TGF α (uncleaved TGF α , which is membrane-bound), may stimulate epidermal growth factor receptors in dysplastic cells by autocrine and paracrine mechanisms, respectively.²⁷

Apoptosis may also be inhibited late in a proportion of dysplastic cells that give rise to invasive or metastatic cells.⁶³ The bcl-2 gene is not overexpressed, as is recognized in colorectal adenomas, although p53 mutations may affect the proliferation/apoptosis ratio in the esophagus.⁶⁴ In addition, up-regulation of immunological death factors such as Fas ligand in the epithelium may not only protect Barrett's dysplastic cells but also may selectively destroy cytotoxic T cells by crosslinking Fas.⁶⁵

In established Barrett's mucosa, identical clonal cytogenetic abnormalities, aneuploidy, and gene amplification⁶⁶ are identifiable in diverse locations. If this is indeed the result of the lateral migration or clonal expansion of transformed clones and not of coincidental oligoclonal or field genetic changes, then catenin-regulated transcription may be partly responsible. Interestingly, recent evidence has shown that this process in Barrett's metaplasia may partly involve down-regulation,⁶⁷ mutation, or phosphorylation of cadherin/catenin adhesion complexes, thereby increasing free cytosolic catenin.^{7,68,69} In addition, the APC gene product (chromosome 5q), which has increasing loss of heterozygosity in the dysplastic progression of Barrett's clones,^{16,47} may lead to reduced β -catenin degradation. Increased β -catenin levels have been shown to subsequently aggregate with transcription

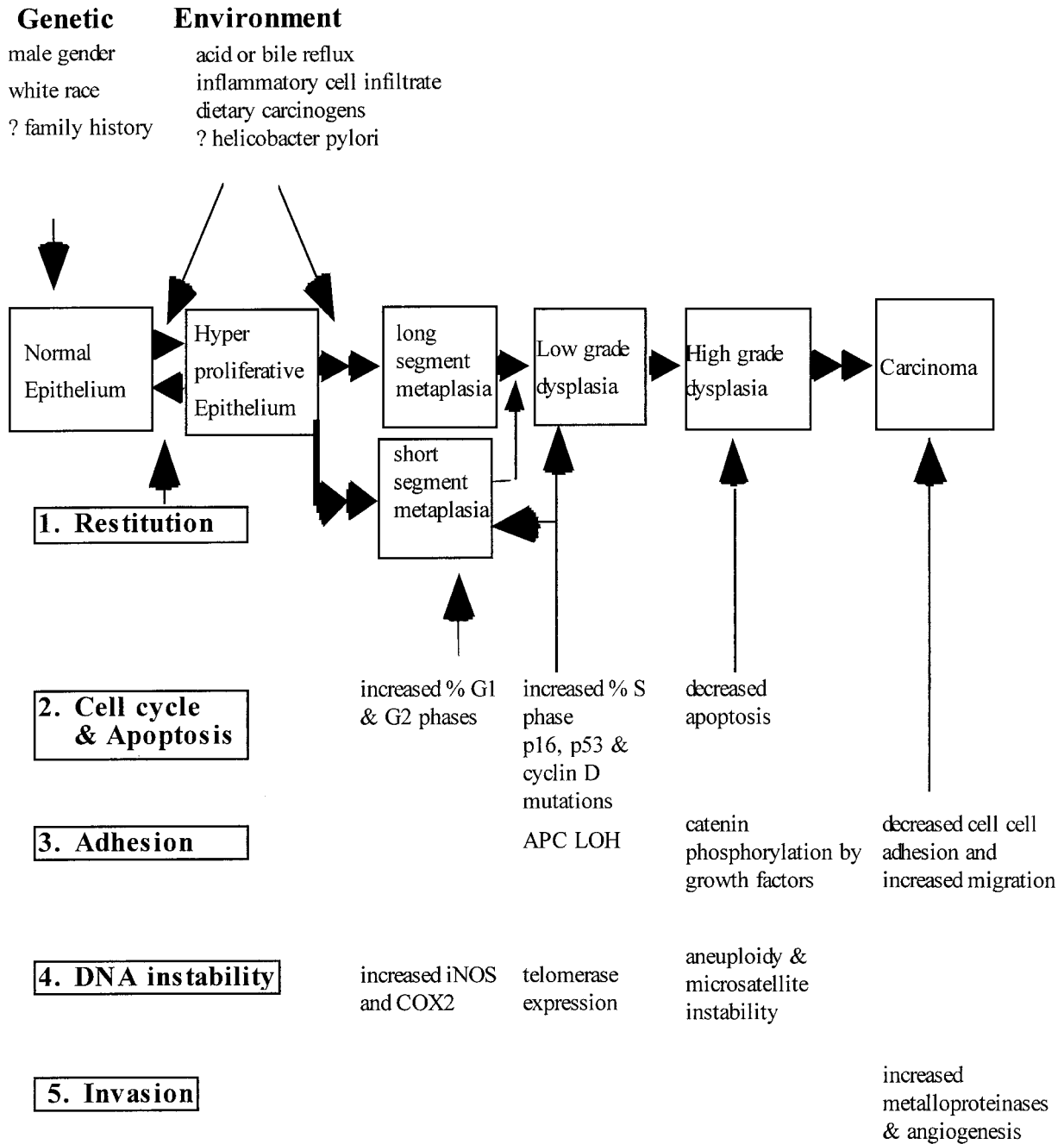


Figure 3. Schematic representation of the key molecular events in Barrett's dysplasia, the metaplasia-dysplasia-adenocarcinoma sequence (MCS). Acid and bile cause acute damage to the esophagus, which is rapidly healed by restitution or cellular replication (stages 1 and 2). In 10% of cases chronic damage to the epithelial stem cells allows rapid clonal replacement by lineages with a growth advantage containing p53 mutations (stage 2). The formation of each type of Barrett's metaplasia is dependent on the stem cell from which it arises as well as the nature of the mucosal microenvironment. Appearance of dysplasia is associated in part with loss of heterozygosity of APC or alterations in the catenins (stage 3). In 1 in 100 cases, aneuploidy and errors in DNA repair represent final pathways which disrupt invasion suppressor genes (stages 4 and 5). The transition from high grade dysplasia to invasive cancer is rapid in all cases.

factors in the nucleus, facilitating epithelio-mesenchymal transition and increased c-myc expression.⁷⁰⁻⁷³

Development of Invasive Adenocarcinoma: Generation of Tumor Heterogeneity and Invasion

The identity of the cell from which esophageal adenocarcinoma originates is speculative because there are con-

flicting data as to whether invasive carcinomas arise from the interactions of multiple oligoclonal lesions (field cancerization) of malignant cells or from a single distinct clone of malignant cells.⁷⁴ In this regard, early neoplasia may be histologically distinct but can be multifocal or immediately juxtaposed with dysplastic tissue. Close but discontinuous dysplastic areas may, however, have different mutations of the p53 gene (chromosome 17p), whereas dysplastic regions contiguous with cancers usu-

ally express identical p53 mutations.^{62,64} Although most research in Barrett's esophagus has focused on mutations occurring in the mutation cluster region in exons 5–8 of the p53 gene, it is conceivable that mutations in other exons or in related proteins could also affect biological function. Interpretation is further complicated by the lack of data concerning the normal clonal colonization patterns of Barrett's crypts such as have recently been noted in the colon and termed patch size.⁷⁵ Available data may suggest that one type of p53 mutation early in the disease is not sufficient to cause adenocarcinoma,⁷⁴ although it seems that p53 mutations accumulate in cancer cells because transformed cells select for specific p53 alterations according to their biological effects. Recently, Barrett's tumors with synchronous high grade dysplasia and invasive cancer were analyzed and showed genetic alterations that were found to be conserved in the synchronous invasive cancers.⁵⁹ These data supported the paradigm of clonal derivation of the invasive cancer from the high grade dysplasia or early invasive cancer. Although these invasive tumors may or may not possess new mutations, a proportion of high grade lesions have genetic abnormalities that may develop but are not present in the synchronous invasive cancer. This heterogeneity indicates genetic divergence during the clonal evolution of cancer, particularly at the time when high grade dysplasia progresses to invasive cancer⁷⁶ (Figure 3). Eight other tumor suppressor gene loci have loss of heterozygosity in Barrett's adenocarcinoma, including VHL (chromosome 3p) in 64%, APC (chromosome 5q) in 45%, CDKN2 (chromosome 9p) in 52%, the retinoblastoma gene (Rb) (chromosome 13q) in 50%, the deleted in colorectal cancer gene (chromosome 18q) in 70%,⁷⁷ and the cgene in 20% of esophageal cancers.⁷⁸ Uncharacterized candidate oncosuppressor gene loci also include 9q (60%), 11p (61%), and 17q (46%).^{76,79} Some gene loss-of-heterozygosity patterns are significantly associated, such as 5q and 9p. Interestingly, the Y chromosome is lost in 9% of Barrett's metaplasia, in 38% of cases indefinite for dysplasia, and in 100% of high grade dysplasia cases, but the significance of this is uncertain because Y chromosomal loss increases with age and in highly proliferating cells.⁸⁰

In a proportion of esophageal adenocarcinomas (5–15%) the phenomenon of ubiquitous microsatellite instability occurs in both diploid and aneuploid Barrett's cell populations, suggesting either that it is an early mutation²⁷ or that these lesions have accelerated the progression to invasive cancer. The genes involved in random error of replication tumors (microsatellite-positive) are similar to colorectal cancer MLH-1 and MSH-2.^{81,82} In addition, these changes may also rarely be associated with transforming growth factor β type II receptor and insulin growth factor type II receptor mutations.^{83,84}

Unifying Molecular Framework and Outstanding Issues Requiring Further Research

In summary, it is postulated that esophageal squamous epithelium adapts to increased chemical damage and

cell loss by acute and chronic responses. In the former, the esophagus increases the growth fraction (number of cells dividing) by hyperplasia and elongation of the proliferative compartment (Figure 2). The chronic response occurs when the initial increase in cell proliferation fails to compensate for the cell loss. There is subsequently selection of specialized lineages of columnar mucosa brought about, in part, by changes in the genotype of the relatively exposed squamous or glandular stem cells. These novel lineages have specific functions including protection against acid, protection against bile (specialized intestinal metaplasia), and repair of ulceration (the ulcer-associated cell lineage). Subsequent mutagenesis and cell cycling abnormalities followed by epithelio-mesenchymal transition may allow invasive Barrett's cancers to develop. The range of esophageal adaptive responses to environmental stimuli is diverse.^{74,77}

This analysis of the molecular biology of BE explains the long latency period of cancer development as multiple genetic events are required, some gene-environment interactions as well as gene-gene interactions, particularly during regeneration. Genetic differences with the ACS such as infrequent APC and Ki-ras mutations may explain the lack of exophytic growth.

Several issues are incompletely elucidated at present. First, the origin of stem cells that give rise to Barrett's metaplasia are unknown. Second, it is not known whether acid or indeed bile reflux is frequently necessary to initiate metaplastic formation. Third, the clonality of metaplastic glands and the tissue patch size (mucosal surface area of contiguous cells arising from the same clone) are unclear. Fourth, the nature of the mechanism governing the expansion of metaplastic glands into the proximal esophagus is ambiguous. Fifth, the natural history of dysplastic glands, especially the more common low grade dysplasia, is a matter of contention. Sixth, we do not know which biological processes are essential determinants of early invasion.

In conclusion, there is a need for improved understanding of the molecular biology of BE, particularly because the premalignant areas are often not visible endoscopically and may occur over a wider surface area compared with colorectal adenomatous polyps. Although no common and simple molecular pathway of progression is evident, we can correlate the pathophysiology with specific molecular alterations (Figure 3). The corollary is that molecular genetics, when applied to histological material, will also increase accurately our knowledge of the natural history of specific lesions found in the metaplasia-dysplasia-adenocarcinoma sequence.

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