Biomarkers of Esophageal Adenocarcinoma and Barrett’s Esophagus

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Abstract

The rising incidence and poor prognosis of esophageal adenocarcinoma in the Western world have intensified research efforts into earlier methods of detection of this disease and its relationship to Barrett’s esophagus. The progression of Barrett’s esophagus to adenocarcinoma has been the focus of particular scrutiny, and a number of potential tissue and serum-based disease biomarkers have emerged. The epidemiology and pathogenesis of esophageal adenocarcinoma are outlined. Tissue biomarkers allowing risk stratification of Barrett’s are reviewed as well as strategies currently being used to discover novel biomarkers that will facilitate the early detection of esophageal adenocarcinoma. Finally, the uses of biomarkers as predictive tests for targeted treatments and as surrogate endpoints in chemoprevention trials are considered.

Introduction

The incidence of adenocarcinoma of the esophagus has risen steadily in both the United States and in Europe over the last two to three decades, whereas the incidence of esophageal squamous carcinoma has remained relatively static (1, 2). Esophageal adenocarcinoma is frequently accompanied by Barrett’s esophagus, a metaplastic condition in which the squamous epithelium lining the lower esophagus is replaced by columnar epithelium, often of a specialized intestinal type. There has also been an apparent increase in the incidence of Barrett’s metaplasia, but because this has paralleled the increasing use of upper gastrointestinal endoscopy, it is not clear if this increase is real or artifactual (3).

Although endoscopic ultrasound and positron emission tomography scanning (4) have improved preoperative staging, most esophageal carcinomas are only detected at an advanced stage and, therefore, this remains a lethal disease with an overall 5-year survival of only 10–20%. The morbidity and mortality associated with the various forms of esophagectomy remain high. Neo-adjuvant chemo- and radiotherapy may have a role in shrinking bulky T4 cancers, improving the chances of complete resection, and providing a survival advantage (5). Refinements in existing treatments are likely to produce no more than incremental improvements in survival. Earlier detection of the cancer (or its premalignant neoplastic precursor) combined with less radical ablative surgical approaches and novel targeted therapies or chemoprevention strategies are likely to realize significant survival benefits.

Although esophageal adenocarcinoma is frequently accompanied by Barrett’s metaplasia, only approximately 5% of patients who present with esophageal adenocarcinoma have an antecedent diagnosis of Barrett’s metaplasia (3, 6, 7). The majority of patients presenting with esophageal adenocarcinoma will therefore not benefit from refinements to endoscopic surveillance programs for Barrett’s metaplasia. The best hope for earlier detection of these cancers lies with the identification of biomarkers that can be assayed in readily obtainable biological samples such as serum or urine from patients who are considered at high risk from an epidemiological perspective.

Epidemiology and Etiology of Esophageal Adenocarcinoma and Barrett’s Esophagus

The rapidly increasing incidence of esophageal adenocarcinoma suggests that environmental influences dominate the etiology. Squamous carcinoma of the esophagus is strongly associated with alcohol consumption and smoking (8) and shows a different geographical distribution to esophageal adenocarcinoma. Smoking and alcohol consumption are also linked to esophageal adenocarcinoma (9), but the association is weaker. Obesity is an important risk factor, but the increasing prevalence of obesity in developed countries is unlikely to be the sole explanatory factor (10, 11). Recent investigations have highlighted the importance of symptomatic gastro-esophageal reflux as a risk factor (12). It has been suggested that reflux of duodenal contents, bile, and gastric acid are also important in the pathogenesis of gastroesophageal reflux disease (13, 14). However, not all patients with reflux develop Barrett’s esophagus, and other factors must also be important. Barrett’s esophagus is particularly prevalent in obese middle-aged Caucasian males. Scleroderma may also be associated with the development of esophageal adenocarcinoma and Barrett’s esophagus (15). Infection with cagA+ strains of Helicobacter pylori (16) and regular use of aspirin (17) or other nonsteroidal anti-inflammatory drugs may reduce the risk.

In addition to these environmental factors, there have been several studies showing that Barrett’s esophagus and esophageal adenocarcinoma can occur frequently within families, suggesting that an inherited risk component may be present (18–20). Interleukin 1β polymorphisms have been associated with increased risk of gastric cancer but not esophageal adenocarcinoma or tumors at the cardia (21), and it has been speculated that similar polymorphisms affecting the inflammatory response might also be important in esophageal adenocarcinoma (22). Inherited polymorphisms affecting genes controlling the metabolism of xenobiotic and carcinogenic compounds are implicated in other cancers, such as those of the lung, and may also prove to have a role in esophageal carcinogenesis. Such approaches may allow identification of subsets of individuals within a population who are predisposed to esophageal adenocarcinoma.

Barrett’s Esophagus and Risk of Esophageal Adenocarcinoma

Barrett’s metaplasia has been reported to increase the risk of developing esophageal adenocarcinoma. Adenocarcinomas develop in Barrett’s esophagus at a rate estimated between 1 cancer per 175 patient years of follow-up to 1 cancer per 441 patient years of follow-up (23, 24). Put in a different way, for adult patients with Barrett’s esophagus, the annual rate of cancer development is approximately 0.4% (25). The rate of cancer development in Barrett’s may also depend on the epidemiological cohort studied: i.e., the rate of...
Invasive adenocarcinoma was present elsewhere in the resection specimen.

Regular endoscopic surveillance has been recommended for patients with Barrett’s metaplasia because these patients are at increased risk of developing adenocarcinoma. It has been shown that cancers detected in such programs are frequently earlier stage and have a better prognosis (30, 7). However, these analyses are subject to confounding factors such as lead and length time bias, and it remains to be established conclusively if surveillance is beneficial. Cost benefit analyses suggest that the cost of detecting an esophageal adenocarcinoma in an endoscopic surveillance program is similar to the cost of detecting breast cancer by mammography (31).

Dysplasia occurring in Barrett’s mucosa is an ominous change and in itself constitutes a biomarker of increased risk of progression to adenocarcinoma. Although some studies have suggested that high-grade dysplasia (Fig. 1) is highly predictive of incipient malignancy (32), there is considerable variation in the reported rates of progression to adenocarcinoma. Between 9 and 59% of patients with high-grade dysplasia on biopsy progress to adenocarcinoma within 5–8 years, and in one study at least, 47% of unifocal high-grade dysplasia regressed (33, 34). The extent as well as severity of dysplastic change may also be important (35, 36). Although specialty pathologists may be able to make reproducible diagnoses of high-grade dysplasia (32), community pathologists only reproduce specialty pathologist diagnoses of high-grade dysplasia about 30% of the time (37). In many centers, once a second pathologist independently confirms high-grade dysplasia, esophagectomy may be performed (38). Approximately 40% of patients with a preoperative diagnosis of high-grade dysplasia have occult carcinoma at resection (39, 40). It has also been suggested that it is possible to follow patients with high-grade dysplasia using surveillance alone (41), but such an approach requires extensive and systematic biopsies to exclude occult synchronous-invasive adenocarcinoma. Other less radical endoscopic ablative approaches have also been tried (42), but the long-term efficacy of such treatments remains unknown, and the use of such treatments should be confined to clinical trials. It has been suggested that esophagectomy remains the standard of care for good surgical candidates with high-grade dysplasia (43).

The risks associated with low-grade dysplasia are even less well-defined (44). It can be difficult to reliably distinguish low-grade dysplasia from regenerative change or atypia accompanying active inflammation, and interobserver variation is high in a community hospital setting (37). Computerized image analysis combined with quantitation of immunohistochemical staining for p53 and Ki67 (45) may reduce interobserver variation and assist in more accurate and reproducible grading, but such an approach has not gained widespread acceptance. In a recent study, 3 of 15 cases of low-grade dysplasia progressed to malignancy (median progression-free survival was 60 months), but progression rates of low-grade dysplasia and indefinite dysplasia were similar (32). Low-grade dysplastic change may apparently regress, and conservative management with endoscopic surveillance is recommended. The identification of dysplastic change in an index biopsy of Barrett’s metaplasia (prevalent dysplasia) may be associated with higher rates of progression than dysplastic change detected in the course of surveillance (incident dysplasia; Ref. 32).

Whatever the precise risk Barrett’s metaplasia confers, multiple studies have shown that the majority of patients do not progress to dysplasia or carcinoma. The vast majority of patients enrolled in surveillance programs do not progress, calling into question the benefit of repeat endoscopies at regular intervals. If it were possible to further stratify the risk of progression of Barrett’s metaplasia to adenocarcinoma, this stratification might permit more effective targeting of repeated endoscopy to patients at particularly high risk of progression. A better understanding of the molecular events surrounding the development of dysplastic change and progression to adenocarcinoma may ultimately help in identifying those patients at increased risk of progression.

**Pathogenesis of Esophageal Adenocarcinoma**

Progression from metaplastic Barrett’s epithelium to invasive adenocarcinoma is a multistep process that may take many years. Specialized intestinal-type Barrett’s mucosa is a highly organized epithelium that may arise by metaplastic change from epithelial stem cells after ulceration of the squamous epithelium (46). A distinct type of multilayered epithelium has been described recently in association with Barrett’s mucosa, with characteristics that are intermediate between squamous and columnar mucosa (47). This epithelium also resembles, and may arise in continuity with, esophageal submucosal gland ducts, and this has led to renewed speculation that the metaplastic epithelium of Barrett’s may arise from submucosal gland ducts (29).

The molecular genetic features of esophageal adenocarcinoma are not as well characterized as those of colonic adenocarcinoma. Although many of the same genes and fundamental cell regulatory systems are involved, there are significant differences between the adenoma-carcinoma sequence in the colon and the proposed metaplasia-dysplasia-carcinoma sequence in the esophagus. There are some similarities with the development of dysplastic change in nonpolypoid flat mucosa and adenocarcinoma in patients with longstanding inflammatory bowel disease (46).

Meticulous mapping studies of the resected esophagus in cases of early adenocarcinoma and high-grade dysplasia have shown large and irregularly shaped areas of dysplastic change (48) with identical cytogenetic abnormalities (49). This suggests lateral migration or clonal expansion of the dysplastic cells. However, there is conflicting data as to whether invasive cancers arise from a single distinct clone of cells or from the interactions of multiple oligoclonal lesions (46). Both models are not mutually exclusive. Each mutation or genetic

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**Fig. 1.** This illustration shows specialized intestinal type mucosa (**top right**) and increasingly severe dysplastic changes amounting to at least severe dysplasia (**bottom left**). Invasive adenocarcinoma was present elsewhere in the resection specimen.
The phosphorylation of Rb and progression to S-phase. p16 may be silenced by hypermethylation of its promoter in esophageal cancer (64). Clones of cells with at least one inactive p16 allele may undergo clonal expansion and constitute early events in this process in the esophagus (69). Cyclin D1 protein overexpression has been documented in Barret’s esophagus and esophageal adenocarcinoma (70).

Abnormalities of p53 are found in both esophageal adenocarcinoma and dysplastic Barret’s mucosa. The mutational spectrum encountered in the TP53 gene in esophageal adenocarcinoma with numerous transitions at CpG dinucleotides (71) is consistent with random mutations secondary to increased cell turnover, as opposed to the signature of chemical carcinogenesis with more frequent transversions (72) seen in esophageal squamous cell carcinoma.

TP53 mutations are found in a majority of esophageal adenocarcinomas and in adjacent high-grade dysplasia, and analyses using cell populations purified by a flow-sorting procedure have shown that the same p53 mutations may be found in diploid cell populations from dysplastic mucosa, from cancer, and from multiple aneuploid cell populations in cancer (56). Inactivation of p53 is known to disable a G1 checkpoint allowing clonal expansion of karyotypically abnormal cells, which evade apoptosis.

Using conventional histological identification of apoptotic bodies, it has been reported that apoptotic indices are reduced in dysplasia and adenocarcinoma compared with metaplastic controls, and there is an increase in the glandular proliferation to apoptosis ratio (73). However, there are conflicting data regarding apoptosis, which may relate at least in part to differing methods of quantifying apoptosis (74, 75). Fas ligand expression is increased in Barret’s metaplasia associated with dysplasia and adenocarcinoma (76). Normal senescence pathways are circumvented in many cancers through de-repression and activation of telomerase, an enzyme that is responsible for stabilization and maintenance of telomere length. Telomerase activity has been demonstrated in esophageal adenocarcinoma using the telomeric repeat amplification protocol (TRAP) assay (77), and increased expression of human telomerase reverse transcriptase mRNA has been demonstrated in metaplastic, dysplastic, and malignant esophageal tissue (78).

Activation of the WNT APC β-catenin pathway in esophageal cancer may occur by a variety of mechanisms but does not play as prominent a role as in colorectal cancer. Loss of heterozygosity at the APC locus has been identified in both dysplastic mucosa and adenocarcinoma but is often preceded by 17p LOH and is not considered a critical or early event (79). However, APC and β-catenin mutations are now recognized to be rare (80–82), and in a mouse knockout model, inactivation of APC was not necessary for the development of esophageal adenocarcinoma (83). There is evidence of reduced expression of E-cadherin (84); this can also lead to increased phosphorylation of β-catenin and translocation to the nucleus, increasing transcription of downstream effectors such as c-myc.

Growth factors and their receptors have also been studied; tumor necrosis factor α shows progressively increased expression with dysplasia and carcinoma and may feed into the WNT1 β-catenin APC pathway described above (85). HER-2/neu protein expression (86) and gene amplification have also been documented. Amplification of the HER-2/neu gene may be detected by a variety of techniques, including PCR (87) or fluorescence in situ hybridization (88). There has been some controversy regarding the frequency and importance of this abnormality in esophageal carcinoma; this may be explained because of different techniques and assays used in the evaluation of this biomarker. With regard to the receptor tyrosine kinase rasraf mitogen-activated protein kinase pathway, activating ras mutations have been detected, but these seem relatively uncommon in comparison with adenocarcinomas of the small and large bowel (89, 90). Recently, in vitro studies have shown that exposure of an esophageal event that carries a selection advantage will result in successive waves of clonal expansion. This process does not necessarily proceed in a simple linear fashion (50). Eventually, the accumulation of sufficient discrete abnormalities completely disrupts normal regulatory mechanisms, resulting in a clone of cells with malignant potential, and completing the progression from inflammation to metaplasia, dysplasia, and adenocarcinoma (51).

The progression to invasive malignancy is also characterized by genetic instability. Dysplastic Barret’s mucosa and esophageal adenocarcinomas often have karyotypic abnormalities, as evidenced by abnormal chromosome number (detected by fluorescence in situ hybridization, interphase cytogenetics; Ref. 52). Abnormalities of chromosomes 1, 8, and Y have been detected (53). Flow cytometry has demonstrated aneuploid DNA content in dysplastic lesions (54) and multiple aneuploid subclones in invasive adenocarcinoma (55). Inactivation of p53 appears to be closely linked to the emergence of aneuploid subclones (56).

Loss of heterozygosity (LOH) has been described at a number of loci in esophageal carcinogenesis. High rates of LOH involving a particular locus suggest inactivation of a tumor suppressor gene at or near the locus. LOH has been demonstrated at 17p, involving the p53 locus (57); at 9p, involving p16 (58); at 5q, involving the adenomatous polyposis coli (APC) locus (59); at 13q, involving the retinoblastoma gene; and at 18q, involving the deleted in colon cancer gene. LOH is now commonly detected using microsatellite polymorphisms, and it is recognized that changes in allelic dosage (allelic imbalance) in such assays are not always associated with tumor suppressor gene inactivation and may also occur secondarily to aneuploidy.

Microsatellite instability has also been demonstrated in esophageal adenocarcinoma and in dysplastic and metaplastic mucosa (60). Microsatellite instability may also be found in sporadic colorectal cancer, but it is more often associated with inheritance of a faulty copy of one of the mismatch repair genes responsible for hereditary nonpolyposis colorectal cancer when it typically involves multiple loci. Although low-level microsatellite instability appears common in esophageal cancer, instability at multiple loci (high-level microsatellite instability) in tumors is rare (61), and hMLH1/hMSH2 protein expression appears conserved in esophageal adenocarcinomas (61). Low levels of microsatellite instability may simply reflect generalized genomic instability rather than specific defects of mismatch repair (62).

Although genetic alterations such as deletions or somatic mutations are widely believed to underlie neoplastic transformation, promoter DNA hypermethylation has also been implicated as an important epigenetic event in tumorogenesis. Hypermethylation of normally unmethylated promoter CpG islands (cytosines located 5′ to guanines) is associated with transcriptional silencing and loss of gene function (63). Examples of relevant hypermethylated genes in esophageal adenocarcinoma include p16(INK4a) (Ref. 64) and APC (familial adenomatous polyposis; Refs. 63 and 65). It has been suggested that some gastric and colorectal cancers show a CpG island phenotype defined by patterns of highly concordant CpG hypermethylation of specific genes (66). Evidence for a CpG island phenotype has not been found in esophageal cancer (67).

Cell cycle regulatory genes implicated in esophageal adenocarcinoma include p16(INK4a), cyclin D1, and TP53. Cyclin D1 is complexed with cyclin-dependent kinases (CDK4/6), and the activated kinases phosphorylate the retinoblastoma protein, Rb. Phosphorylated Rb can no longer bind and repress the E2F transcription factors, allowing progression to S-phase of the cell cycle. p16 forms p16-CDK4/6 complexes preventing the formation of cyclin D1/CDK4/6 complexes. Inactivation of p16 or overexpression of cyclin D1 favors the phosphorylation of Rb and progression to S-phase. p16 may be inactivated through mutation or deletion (68), or its expression may be decreased by hypermethylation of its promoter in esophageal cancer (64).
adenocarcinoma cell line to acid can switch on the mitogen-activated protein kinase pathways, promoting proliferation and inhibiting apoptosis (91).

Cyclooxygenases (COX) are enzymes that mediate the production of prostaglandins from arachidonic acid. COX-2 is detectable in metaplastic Barrett’s mucosa and is overexpressed in dysplasia and esophageal adenocarcinomas (92, 93). COX-2 may have effects on cell proliferation, apoptosis, and angiogenesis. The activity of COX-2 may be inhibited by existing selective COX-2 inhibitors, further increasing the importance of this enzyme as a potential biomarker (see below). COX-2 may also promote angiogenesis through its induction of vascular endothelial growth factor (94, 95).

**Biomarkers**

A biomarker may be defined as a characteristic that is measured or evaluated as an indicator of pathological processes or a response to a therapeutic intervention. An ideal biomarker of malignancy will show variation in expression associated with the process of neoplastic transformation and will be detectable early in a premalignant phase. The discovery and evaluation of cancer biomarkers represents a very complex task necessitating multidisciplinary collaboration between epidemiologists, basic scientists, clinicians, and industry. The National Cancer Institute has recently formed the Early Detection Research Network (96) to facilitate this process, and this group has suggested that the process may be divided into five phases analogous to the clinical trial structure used in testing new drugs (97). Such a structure also helps in the evaluation of published biomarker studies.

**Endoscopic Biomarkers of Dysplasia and Malignancy**

Most esophageal adenocarcinomas are diagnosed by endoscopy and biopsy. It will be apparent that there is often considerable tissue heterogeneity in the lower esophagus harboring an occult early cancer. The diagnosis of early lesions may be difficult, because the endoscopist may not be able to recognize dysplastic areas of columnar mucosa or early cancer. Rigorous, systematic biopsy protocols may be able to distinguish between early invasive adenocarcinoma and high-grade dysplasia (41), but in many centers where such protocols are not rigidly adhered to, representative sampling remains a significant issue. It has been suggested that endoscopically visible ulceration of the mucosa is a useful biomarker of malignancy (98). Endoscopic ultrasound has emerged as a technique for the preoperative assessment of T and N staging. Endoscopic ultrasound with a high frequency probe can also detect areas of mucosal thickening and therefore assist in the detection of some early lesions (99). Another approach has been to use methylene blue staining (100) or fluorophores such as 5-aminolevulinic acid (101) to visualize dysplastic or neoplastic tissue more readily at endoscopy.

**Tissue Biomarkers**

Tissue biomarkers that have been evaluated by immunohistochemistry include cyclin D1, p53, and markers of cell proliferation. It has been recognized for some time that the cell cycle is dysregulated in dysplastic Barrett’s mucosa with increased Ki67-labeling indices. Moreover, there is evidence of loss of spatial organization, with abnormal expression of Ki67 on the surface epithelium in high-grade dysplasia (102).

Immunohistochemical detection of p53 shows a higher fraction of positively staining adenocarcinomas (87%) compared with dysplastic (9–55%) or metaplastic (0%) mucosa, and frequently there is evidence of topographical colocalization of the positive staining with dysplastic change in biopsies (103). It has been proposed that the combination of p53 protein expression and disordered proliferative architecture may be used as an objective biomarker to assist in the recognition and diagnosis of dysplastic change (104, 105).

Tissue biomarkers may also be used directly to stratify the risk of progression. It has been reported that p53 protein expression colocalized to low-grade dysplasia (Fig. 2) conferred an increased risk of progression to multifocal high-grade dysplasia or adenocarcinoma (106, 107). Cyclin D1 expression in nondysplastic Barrett’s has also been associated with an increased risk of progression to adenocarcinoma, but in one phase 3 case-control study, p53 protein expression did not confer an increased risk (108), and in fact, 69% of the patients progressing to cancer had negative p53 immunostaining.

Aneuploid DNA content as detected by flow cytometry has been used as an objective tissue biomarker that is associated with progression to dysplasia. Multiple aneuploid subpopulations were associated with the development of invasive malignancy. In a study from the Seattle group, no patients progressed without some evidence of abnormal DNA content on flow cytometry (35, 109). The same group has also evaluated loss of heterozygosity of chromosome 17p as a biomarker of progression (110). This also proved to be an excellent predictor of progression. Although it was suggested that 17p LOH reflects p53 inactivation, it is possible that such an assay reflects inactivation of other tumor suppressors at this locus or genome-wide instability. Detection of mutation by PCR and direct sequencing or using a screening technology such as single-strand conformation polymorphism analysis would constitute a more specific test of p53 inactivation.

Evaluation of p53 protein expression or mutation or allelic loss as a biomarker is complicated by the complex relationship between p53 mutation, deletion, and protein expression. Approximately 30–35% of esophageal adenocarcinomas harbor nonsense or frameshift mutations (111–113) that do not result in the accumulation of mutant protein and that appear as false negatives using an immunohistochemical staining assay. Moreover, not all positively staining cases have been shown to harbor mutations (114).

**Fig. 2.** A group of crypts shows strong p53 protein expression. Note negatively stained metaplastic glands (right) and also some negatively stained dysplastic glands (left). Immunoperoxidase, hematoxylin counterstain.

Brush cytology has been used as an adjunct to conventional biopsy and histopathology (115). Currently, few pathologists would advocate the use of cytology in this clinical context. However, brush cytology has the potential to sample tissues more widely than endoscopic biopsy. Improved methods of cytological specimen preparation and immunocytochemistry using monoclonal antibodies to novel tissue biomarkers (116) may yet lead to a useful role for cytology.
Villin is a Ca\(^{2+}\)-dependent actin-binding protein that is required to form the apical microvilli found in normal intestinal epithelium and is strongly and frequently expressed in Barrett’s mucosa, esophageal adenocarcinoma, and tumors of the gastric cardia (117). Immunocytochemistry for villin protein may assist in the cytological identification of intestinal metaplasia and lead to more sensitive and specific diagnosis of Barrett’s metaplasia (118). Immunocytochemistry for markers such as p53, p16, or cyclin D1 might facilitate early detection of neoplastic clones in dysplastic mucosa before clonal expansion and progression. Technologies such as laser scanning cytometry may be applicable, allowing measurement of DNA content or quantitation of fluorescence in situ hybridization signals without the need for tissue disaggregation. The focality of dysplastic change in esophageal carcinogenesis represents a challenge to the detection of somatic mutations and other genetic abnormalities in biopsy or cytology specimens. However, a similar situation applies to the detection of such abnormalities in other cancer types and sites. Sensitive methodologies are needed to detect mutations or similar abnormalities in small numbers of cells in precursor lesions but not so sensitive as to generate large numbers of false-positive signals.

Methylation-specific PCR represents an exquisitely sensitive technique to test for low abundance-methylated sequence. Such an approach has been used to identify focal involvement of prostate needle core biopsies by carcinoma and to detect methylated sequences in urine, serum, and sputum. Surveys of the pattern of gene methylation in esophageal carcinogenesis have shown that normal or metaplastic tissues from patients with associated dysplasia or cancer had a significantly higher incidence of hypermethylation than similar tissues from patients with no evidence of progression (67). Thus, detection of hypermethylation might be a useful approach to the identification of Barrett’s patients at increased risk of progression.

Serum and Urine Biomarkers of Esophageal Adenocarcinoma and Barrett’s

Only a minority of patients presenting with esophageal adenocarcinoma have an antecedent biopsy diagnosis of Barrett’s (6), and therefore, improved risk stratification in Barrett’s is unlikely to result in significant reductions in mortality from esophageal adenocarcinoma at a population level. The development of robust biomarker assays applicable to blood or urine samples might assist in the stratification of risk in patients with symptoms of gastro-oesophageal reflux disease and the selection of patients for endoscopy of the upper gastro-intestinal tract.

Villin has been found in the serum of about 50% of colon cancer patients and represents a useful marker to detect cancer recurrence after tumor resection (56). Autoantibodies to villin were also detected in 80% of patients. Although the highest level of autoantibodies to villin was present in the cancer patients, these autoantibodies were also found in patients with inflammatory bowel disease and in some controls as well. The antigenicity of villin may result from the exposure of the villin protein, which is normally intracellular, after cell lysis occurring spontaneously in tumors or secondary to inflammation. Determination of serum villin levels or detection of villin autoantibodies may assist in the identification of a subset of patients with Barrett’s metaplasia or adenocarcinoma in a population with symptoms of gastroesophageal reflux disease.

Mutations of the p53 gene frequently accompany neoplastic transformation of Barrett’s esophagus and can be detected in dysplastic mucosa. Missense mutations give rise to mutant proteins that frequently have increased stability and that can frequently be detected by immunohistochemistry. Antibodies to p53 can also be detected in the serum of patients with esophageal adenocarcinoma, squamous carcinoma, and Barrett’s esophagus. Such antibodies may also be detected before the development of invasive malignancy (119) and therefore represent another possible biomarker, the role of which in early detection remains to be explored.

The APC gene becomes abnormally methylated in Barrett’s metaplasia and in esophageal adenocarcinoma. Abnormal methylation is also detectable in plasma DNA. Hypermethylation of APC in plasma DNA may be an indicator of aggressive disease in patients with esophageal carcinoma (59).

Biomarker Discovery

The identification of genes involved in the process of carcinogenesis has been a major focus of cancer research over the last two decades. In the post-genomic era, the discovery process is dominated by high-throughput highly parallel technologies such as transcriptional profiling or proteomics. The transcriptional profile of a tumor or a tissue may be determined by hybridization of tumor mRNA to a cDNA array on a glass slide. Such in silico analysis allows simultaneous determination of the relative expression levels of thousands of key genes in tumor tissue. The volume of data produced by such analyses is vast and difficult to interpret, necessitating the use of new bioinformatics approaches.

Transcriptional profiling has been used to study a variety of human cancers. Hierarchical cluster analysis has been used to classify histologically homogenous cancers into different groups with different clinical outcomes based on gene expression patterns. Transcriptional profiling of cancer may permit the development of molecular taxonomy, and such molecular classifications may have a complementary role to conventional histopathological diagnosis and classification. Transcriptional profiling (120, 121) and novel methods of data analysis have been applied recently to esophageal carcinoma and Barrett’s...
esophagus (Fig. 3). This approach has been used to select 160 genes differentially expressed between Barrett’s esophagus and esophageal adenocarcinoma that can be used to distinguish between the two conditions (Fig. 4; Ref. 122). Such genes and gene expression patterns are candidates for further study as tissue biomarkers of neoplastic progression.

Biomarkers as Predictive Tests for Conventional or Targeted Treatments and as Intermediate Endpoints in Cancer Chemoprevention Trials

Biomarkers may also serve as predictive tests for targeted treatments for cancer or for premalignant conditions. COX-2 expression has been reported in both colorectal adenomas and adenocarcinomas and in Barrett’s metaplasia, dysplastic mucosa (Fig. 5), and esophageal adenocarcinoma. COX-2 expression has been reported as an independent adverse prognostic indicator in esophageal adenocarcinoma (123), and an argument can therefore be made for trials that test the efficacy of COX-2 inhibitors as adjuvant-targeted treatments. In animal models of gastro-esophageal reflux, COX-2 inhibition reduces the rate of development of adenocarcinomas (124), and inhibition of COX-2 in vitro reduces the proliferation of Barrett’s cell lines in tissue culture (125) and in vivo in Barrett’s (126). A phase II trial evaluating the selective COX-2 inhibitor celecoxib as a targeted chemopreventive treatment in esophageal carcinoma is reportedly underway.

Biomarkers may also be used as intermediate or surrogate endpoints in cancer chemoprevention trials. A rare or distal endpoint (cancer incidence) may be replaced by a more frequent, proximal intermediate endpoint (resembled by the biomarker), allowing reduction in sample size and/or a shorter trial duration. However, studies based on surrogate endpoints are inherently less reliable than studies with true endpoints, and it is important to have robust criteria by which to judge the suitability of the endpoint (127). In National Cancer Institute sponsored chemoprevention trials, prevention or regression of intraepithelial neoplasia is the typical primary endpoint. However, it is generally held that not all intraepithelial lesions progress and that some, particularly low-grade lesions, may regress spontaneously. It is therefore critical to ensure that chemopreventive treatments target those lesions that would have progressed to invasive cancer. This might be achieved by documenting similar accumulations of genetic lesions within intraepithelial lesions in both the treatment- and placebo-controlled arms of the trial (128).

Recently, there have been anecdotal reports of colorectal adenomatous polyps regressing with celecoxib treatment, but in a clinical trial in patients with familial adenomatous polyposis, there was no evidence of a significant reduction in polyp formation (129). In familial adenomatous polyposis, the polyp burden represents an easily visible and quantifiable phenotypic response that can be used to quantify the response to treatment. However, in the esophagus, matters are more difficult because areas of dysplastic change are not always readily identifiable at endoscopy (or indeed in esophagectomy resection specimens) and are often focal, potentially leading to sampling error. Moreover, interobserver variation in the diagnosis of dysplastic change might also present difficulties. Systematic endoscopic mapping and biopsy or sampling of the resected esophagus with rigorous morphometric and quantitative evaluation of dysplastic change and biomarker expression will be needed to accurately assess the response to chemopreventive treatments (130, 131).

Conclusion

Many studies have evaluated a range of putative tissue biomarkers that might assist in the stratification of the risk of progression of Barrett’s metaplasia to adenocarcinoma. Currently only DNA content as measured by flow cytometry and 17p allelic imbalance represent biomarkers that are prospectively predictive of progression, but such results require verification in large multicenter trials. There have been conflicting reports about the efficacy of p53 immunostaining, and it is clear that p53 mutation is not always accompanied by protein overexpression. No biomarker has yet emerged that is superior to histological identification of dysplasia. The wide variation in the reported rates of progression of dysplasia to malignancy and the problems associated with the reproducibility of such a diagnosis are strong arguments to continue the search. It remains to be seen whether high-throughput hypermethylation analyses (66) or transcriptional profiling (115) can prospectively identify molecularly distinct but histologically indistinguishable high-risk groups of BM patients. Fewer studies have evaluated blood or urine biomarkers, but such approaches could play an even more important role in the early detection of esophageal adenocarcinoma. Biomarker discovery programs are increasingly inextricably linked to the search for novel targeted treatments and to chemoprevention.

References


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