Clinical Use of p53 in Barrett’s Esophagus

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Abstract

Barrett’s esophagus is an established precursor to esophageal adenocarcinoma. Whereas most patients with Barrett’s esophagus do not progress to adenocarcinoma, patients with progression have a poor prognosis. Current management strategies use frequent endoscopic surveillance and multiple nontargeted biopsies. This approach, however, may miss dysplastic areas. Furthermore, given the relatively high prevalence of Barrett’s esophagus but low incidence of progression, this invasive and expensive approach has not been shown to be cost-effective. Thus, there is intense interest in using biomarkers to identify patients at increased risk of progressing to adenocarcinoma. This has included examination of mutations in the tumor suppressor gene, p53. In this report, we discuss the biology of p53 and the incidence of p53 mutations in Barrett’s esophagus and review relevant studies regarding the ability of p53 to predict neoplastic progression. Additionally, we report our results of the expression of p53 by immunohistochemistry in a group of 18 patients that have undergone endoscopic esophageal mucosal resection for dysplasia. Although the presence of a p53 mutation increases the risk of neoplastic progression, the absence of this mutation does not abrogate the risk. Continuing efforts, therefore, are needed to define and prospectively validate a panel of biomarkers to risk-stratify patients with Barrett’s esophagus. Determination of p53 mutational status may ultimately be a component of such a panel. (Cancer Epidemiol Biomarkers Prev 2006;15(7):1243–9)

Introduction

In Barrett’s esophagus, the normal esophageal squamous epithelial lining is replaced with specialized intestinal metaplasia. Endoscopically, this change appears as red velvety mucosa extending above the gastroesophageal junction (1-3). Barrett’s metaplasia is a premalignant condition and represents the precursor to esophageal adenocarcinoma. The incidence of esophageal adenocarcinoma in the United States has been rising dramatically at a rate faster than any other malignancy (4). Identified risk factors for the development of Barrett’s esophagus include gastroesophageal reflux disease, Caucasian ethnicity, male gender, and older age (3, 5).

Although endoscopic mucosal features may suggest the presence of Barrett’s esophagus, the diagnosis currently must be confirmed histologically via endoscopic biopsies. Additionally, although the presence of Barrett’s esophagus predisposes to the development of esophageal adenocarcinoma, progression is by no means inevitable (6). Currently, in the absence of high-grade dysplasia or cancer, surveillance endoscopies are recommended at regular intervals to sample areas of Barrett’s esophagus for neoplastic progression (1). However, the cost-effectiveness and even the utility of such a strategy has been debated in the literature (5, 7). For these reasons, strategies to stratify groups at high risk for development of adenocarcinoma are needed. Studies have suggested that certain molecular biomarkers, including p53 mutations, may aid in this risk stratification. Furthermore, an understanding of intermediate molecular biomarkers may aid in the development and testing of pharmaceutical agents in cancer chemoprevention trials (8, 9). The biology of p53, its role in cancer biology, and studies using p53 in Barrett’s esophagus to predict clinical outcome and guide surveillance will be reviewed in this context.

Pathogenesis of Barrett’s Esophagus and Esophageal Adenocarcinoma

Metaplasia is the transformation of one differentiated adult cell type into a different mature cell type. Barrett’s metaplasia is the replacement of the normal stratified squamous epithelium of the distal esophagus by a specialized columnar epithelium possessing both columnar cells and goblet cells (intestinal metaplasia; ref. 10). The transformation from squamous mucosa to columnar mucosa seems to be an adaptive response to chronic reflux disease as the columnar mucosa is more resistant to acid injury (11). Barrett’s esophagus, however, may also be thought of as a potential premalignant neoplastic process because it is associated with clonal expansion and progression (12).

As in other epithelial cancers, esophageal adenocarcinogenesis proceeds through a multistep neoplastic progression. The proposed pathway progresses from normal squamous mucosa to esophagitis to Barrett’s metaplasia to dysplasia to adenocarcinoma (11). A neoplastic cell may be defined as one that has clonally expanded as a result of somatic mutations (13). For this reason, some authorities have recommend the use of the term intraepithelial neoplasia rather than dysplasia, as this classification more closely reflects the malignant potential of the condition (10). Dysplasia is commonly subclassified into indefinite, low-grade, and high-grade categories. This classification is based only on morphologic and not molecular features and suffers from interobserver variability (14-16).

Hanahan and Weinberg proposed six hallmarks essential to cancer development (17): (a) self-sufficiency in growth signals, (b) insensitivity to growth-inhibiting signals, (c) evasion of apoptosis, (d) limitless replicative potential (avoidance of senescence), (e) sustained angiogenesis, and (f) tissue invasion and metastasis. The presence of these hallmarks in the cancer biology of Barrett’s esophagus was reviewed by Morales et al.
in 2002 (11). More broadly, these changes can be summarized as increased proliferative activity, prolonged life span, and invasive and metastatic capacity. Because p53 regulates proliferation via G1-S check point control and also functions in the control of apoptosis, loss of p53 function can be critical for tumor progression (18).

**Role of p53 in Control of Cell Cycle and Cell Death Pathways**

The p53 gene encodes a protein of 53 kDa (thus p53). This gene, first identified as a tumor suppressor gene in 1979, is located on chromosome 17p (18, 19). Wild-type p53 regulates diverse cell functions, including cell cycle progression, senescence, differentiation, and apoptosis (ref. 20; Fig. 1). Recently, the role of p53 in DNA repair after genotoxic insult has also been described (21). The most common responses to oncogenic stress induced by p53 are cell cycle arrest and DNA repair. If the DNA damage cannot be repaired, p53 induces apoptosis (20, 22).

The p53 gene product is normally “off” and is “activated” by post-translational modifications, such as phosphorylation, in response to cellular stress or damage. Multiple stresses lead to p53 activation, including DNA damage, aberrant growth signals (dependent on cyclin-dependent kinase inhibitor p14), treatment with chemotherapeutic agents, exposure to UV light, and protein kinase inhibitors (23). These cellular stresses lead to increased p53 protein expression (mainly through decreased degradation). Increased wild-type p53 inhibits cellular division and/or induces apoptosis.

**Mechanisms of p53 Loss of Function**

Given the diverse roles played by p53 to protect the integrity of cellular DNA, it is not surprising that p53 loss of function predisposes to the development of cancer (24). Mutations of the p53 gene are the most prevalent genetic lesions in human cancers, occurring in at least 50% of tumors (25). Approximately 90% of the mutations in p53 are point mutations (20). Many p53 mutations are thought to arise by oxidative damage to cytosine bases in exons 5 to 8 (26). Acid suppressive therapies may decrease oxidative DNA damage, although preceding p53 mutations seem to prevent this reduction (27). Many of these mutations encode changes that stabilize the protein, preventing its breakdown and thereby leading to increased levels of mutant p53 within the cell. Because p53 functions as a tetrameric complex and the p53 mutations commonly preserve protein-protein interactions, mutant p53 can function as a dominant-negative inhibitor (competitive inhibition) of wild-type p53 (28).

Dysregulation of proteins that control p53 can also cause loss of p53 function (23). MDM2, the major negative regulator of p53, targets p53 for degradation. MDM2 is itself transcriptionally up-regulated by p53. Thus, p53 and MDM2 form an autoinhibitory loop. Hence, MDM2 down-regulation can increase p53 expression, whereas MDM2 overexpression can inhibit p53 function, increasing the susceptibility of the cell to oncogenic stresses. Derangements in MDM2 regulation, including MDM2 overexpression, are observed in some tumors with wild-type p53, including esophageal adenocarcinoma (29, 30).

**Relationship between p53 Mutations and Immunohistochemical Expression**

Many p53 mutations increase the protein half-life, leading to increased levels of protein expression. Thus, mutant p53 can often be readily detected by immunohistochemistry. In contrast, wild-type p53 has a short half-life in the cell and is usually present at levels below the threshold of detection by immunohistochemistry (31). Some p53 mutations, however, produce a truncated protein that is not detectable by immunohistochemistry (32-35).

In a study of 17 esophageal adenocarcinoma specimens, 16 p53 mutations were identified in 15 cases (36). These tumors were then examined by immunohistochemistry. Five (31%) cases with p53 mutation did not have detectable protein by immunohistochemistry. These were all chain-terminating mutations that produced a truncated p53 protein.

In a study by Coggi et al. of 74 patients who underwent esophagectomy for malignancy (both adenocarcinoma and squamous), p53 gene mutations as assessed by single-strand conformational polymorphisms were detected in 39 (53%) carcinomas (37). In 12 (31%) of these cases, there was no detectable p53 accumulation by immunohistochemistry. Sequence analysis was not done in this study, but the authors postulated that some of these cases were due to mutations that produced truncated p53 protein.

Inflammation, DNA damage, and other cellular stresses can up-regulate wild-type p53. For this reason, p53 overexpression does not always correlate with p53 gene mutations (36, 38, 39). In the above study from Coggi et al., 35 tumors without p53 mutation were identified. However, 9 (26%) of these cases were strongly positive for p53 by immunohistochemistry. A more recent study found that whereas 19 of 29 adenocarcinomas strongly expressed p53 by immunohistochemistry, only 2 of these tumors had p53 mutations (26). Thus, it is clear that not all p53 mutations result in p53 protein accumulation and not all p53 protein accumulation is due to a p53 mutation. Despite these limitations, investigators have used p53 overexpression as a biomarker for malignant potential.

**Clinical Use of p53 in Barrett’s Esophagus**

Numerous molecular changes have been identified in the literature in the malignant progression of Barrett’s esophagus. Only some of these changes are likely to play causal roles in Barrett’s carcinogenesis. Other molecular abnormalities may be random events or epiphenomena of other causally important alterations (12). Although these changes may not drive neoplastic transformation, they may still serve as useful

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**Figure 1.** Tumor suppressor p53 is activated by several cell stressors and can respond in a variety of ways, including apoptosis, if significant damage to the cell occurs. Adapted with permission from ref. 20.

**Clinical Use of p53 in Barrett’s Esophagus**

Numerous molecular changes have been identified in the literature in the malignant progression of Barrett’s esophagus. Only some of these changes are likely to play causal roles in Barrett’s carcinogenesis. Other molecular abnormalities may be random events or epiphenomena of other causally important alterations (12). Although these changes may not drive neoplastic transformation, they may still serve as useful
clinical biomarkers if they are closely associated with and predictive of malignant progression. Additionally, for a molecular marker to be clinically useful in surveillance, it must occur before the development of carcinoma (i.e., during the phase of metaplasia or dysplasia).

Biological markers have a broad range of applications (40). In Barrett’s esophagus, a biomarker is most useful as an indicator of disease prognosis. Tockman et al. described four criteria needed for a biomarker to be clinically useful: (a) the marker must be biologically appropriate (i.e., expressed in clinically accessible tissue); (b) it must be possible to establish quantitative values/criteria for the presence or absence of the marker; (c) the biomarker must be validated against acknowledged disease end points; and (d) the biomarker must have a predictive value confirmed in prospective studies (41). With regard to these criteria, p53 expression is easily studied via immunostaining of accessible esophageal mucosal tissue. As noted previously, p53 levels are generally increased with biopsies showing invasive cancer by histology were p53 positive. Forty patients had biopsy specimens containing high-grade dysplasia and 8 (57%) of these were p53 positive. Both patients with biopsies showing invasive cancer by histology were p53 positive. These results are summarized in Table 1. These data suggest that p53 mutations are relatively uncommon in low-grade dysplasia, a finding similar to that reported by previous investigators. Furthermore, results from this population of patients and others suggest that p53 mutation may play a role in the transition from low-grade dysplasia to high-grade dysplasia.

Galipeau et al. showed that inactivation of p53 by mutation is strongly associated with progression to aneuploidy possibly through the loss of p53-mediated apoptosis and cell cycle arrest (56). The development of these aneuploid cell populations has been shown to increase the risk of developing adenocarcinoma (57, 58). Because p53 mutations are more frequent in advanced histology and also possibly participate causally in tumorigenesis, investigators began testing the hypothesis that p53 expression could predict progression to adenocarcinomas (59-66). These studies are summarized in Table 2.

Yonnes et al. studied p53 accumulation via immunohistochemistry in Barrett’s metaplasia, dysplasia, and adenocarcinoma (59). Supporting the results of previous investigators, they found that p53 accumulation increased as histology

Studies of p53 and Barrett’s Esophagus

The first report of p53 gene mutations in esophageal squamous cell cancer appeared in 1990, and 1 year later, there were similar reports of p53 mutations in esophageal adenocarcinomas (42-44). Multiple early studies showed that p53 overexpression is increased as histology progresses from metaplasia to high-grade dysplasia and to adenocarcinoma (45-47). Later studies showed that esophagectomy specimens containing adenocarcinoma with overexpression of p53 had adjacent dysplastic epithelium overexpressing p53 (48, 49). In addition, in many cases, the same p53 mutations are found in the adenocarcinoma and adjacent dysplastic epithelium (50, 51). This suggested that the p53 mutation was an important step in the progression toward adenocarcinoma. Although p53 mutations are common in adenocarcinoma, they are relatively uncommon in nondysplastic Barrett’s esophagus (52).

At the University of Chicago, we determined the expression of p53 in 18 patients that had undergone endoscopic mucosal resection (EMR) for Barrett’s esophagus. EMR is a unique endoscopic procedure in that it allows the removal of all abnormal tissue and retrieval of this tissue for histopathologic analysis. In contrast to surgical esophagectomy, however, EMR preserves the esophagus (53, 54). Barrett’s esophagus in a given patient may exhibit multiple histologic grades of progression and the entire spectrum can be captured by EMR. Immunohistochemical analysis was done as described previously (55). All slides were examined by a single experienced gastrointestinal pathologist (A.N.) and p53 positivity was defined as intense nuclear staining in at least 5% of nuclei (Fig. 2). Five patients had biopsy specimens containing low-grade dysplasia, none of which were p53 positive. Fourteen patients had biopsy specimens containing high-grade dysplasia and 8 (57%) of these were p53 positive. Both patients with biopsies showing invasive cancer by histology were p53 positive. These results are summarized in Table 1. These data suggest that p53 mutations are relatively uncommon in low-grade dysplasia, a finding similar to that reported by previous investigators. Furthermore, results from this population of patients and others suggest that p53 mutation may play a role in the transition from low-grade dysplasia to high-grade dysplasia.

Table 1. p53 status in biopsies from EMR

<table>
<thead>
<tr>
<th>Histology</th>
<th>No. patients</th>
<th>p53 positive, n (%)</th>
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<tbody>
<tr>
<td>Barrett’s esophagus without dysplasia</td>
<td>9</td>
<td>1 (11)</td>
</tr>
<tr>
<td>Low-grade dysplasia</td>
<td>5</td>
<td>0 (0)</td>
</tr>
<tr>
<td>High-grade dysplasia</td>
<td>14</td>
<td>8 (57)</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>2</td>
<td>2 (100)</td>
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</tbody>
</table>

*A patient is defined to be p53 positive for a given histology if at least one biopsy shows intense nuclear staining for p53. Patients may have several different histologies in a single EMR specimen.

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<tbody>
<tr>
<td><em>P</em> &lt; 0.05;</td>
<td>compared with biopsies containing low-grade dysplasia or no dysplasia.</td>
</tr>
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</table>
progressed along the metaplasia-dysplasia-adenocarcinoma sequence in 54 patients (0%, 9%, and 87% for no dysplasia, low-grade dysplasia, and adenocarcinoma, respectively). Follow-up biopsies were available in 23 patients who had dysplasia in at least one biopsy specimen. Only 1 of 21 (4.8%) patients with all p53-negative biopsies had histologic progression. In contrast, 2 of 3 (67%) patients with p53-positive biopsies progressed to high-grade dysplasia or intramucosal carcinoma (one patient lost to follow-up). Thus, their retrospective data suggested that p53 accumulation increased the risk of progression from low-grade to high-grade dysplasia.

In a larger study from the same group, the biopsies from 61 patients with Barrett’s metaplasia or low-grade/indefinite dysplasia were retrospectively examined for evidence of p53 protein accumulation (60). Biopsy tissue from 9 of 25 (36%) patients who initially had or later developed low-grade/indefinite dysplasia during the study period expressed p53 by immunohistochemistry. Five of 9 (56%) of these patients with p53 accumulation and low-grade/indefinite dysplasia developed high-grade dysplasia/adenocarcinoma in follow-up. In contrast, 0 of 16 (0%) patients without p53 accumulation developed high-grade dysplasia/adenocarcinoma (P < 0.01). The mean follow-up for both groups was ~2 years. In this study, p53 accumulation had a higher positive predictive value than low-grade/indefinite dysplasia for progression to high-grade dysplasia/adenocarcinoma.

A retrospective study from Spain also examined the association of p53 expression with histologic progression in Barrett’s esophagus (61). In the 25 patients with Barrett’s metaplasia (no dysplasia), none was found to have p53 overexpression by immunohistochemistry. However, 12 (48%) of these patients had histologic progression. In the group with biopsies indefinite for dysplasia, 6 of 17 were p53 positive. In that group, statistically more patients who were p53 positive progressed compared with those who were p53 negative (80% versus 18%; P = 0.021). In the group with low-grade dysplasia, 2 of 4 patients with p53 positivity progressed, whereas none of the 2 patients negative for p53 progressed. In that study, all patients with high-grade dysplasia were p53 positive. Thus, p53 status was not effective in predicting histologic progression in patients with metaplasia or low-grade dysplasia but did seem to be effective in predicting histologic progression in patients with biopsies indefinite for dysplasia or with low-grade dysplasia. It should be noted that most investigators combine indefinite and low-grade dysplasia due to difficulty in distinguishing between the two histologically (67).

The Cleveland Clinic retrospectively analyzed 16 patients with Barrett’s esophagus and histologic diagnosis of low-grade dysplasia (62). Immunostaining for p53 was done on each of these biopsies. In 9 of 16 (56%) patients, p53 staining was positive in areas with morphologic changes of low-grade dysplasia. Of these, 7 of 9 patients progressed to high-grade dysplasia/adenocarcinoma, whereas 1 of 7 patients negative for p53 staining progressed to adenocarcinoma. This difference was statistically significant (P = 0.017). Thus, in this study, p53 positivity was found to be complementary to histologic diagnosis of low-grade dysplasia in predicting progression to adenocarcinoma.

The above retrospective studies showed an increased expression of p53 in biopsies with high-grade dysplasia or adenocarcinoma compared with biopsies showing only metaplasia or low-grade dysplasia. These analyses, moreover, suggested that p53-positive immunostaining is associated with an increased risk of histologic progression.

More recently, the correlation of p53 expression and Barrett’s progression was evaluated in a prospective manner. Weston et al. prospectively studied 48 patients with low-grade dysplasia to determine whether p53 immunoreactivity predicted histologic progression to high-grade dysplasia/adenocarcinoma (63). Five (10.4%) patients had histologic progression during the study period. The presence of p53 immunoreactivity was significantly associated with risk of progression, but the majority of p53-positive patients did not progress during the follow-up period, and some even regressed. The authors suggested that patients with p53 positivity and low-grade dysplasia might be candidates for a more intensive surveillance program.

In a prospective surveillance study of 307 patients with Barrett’s esophagus, 12 patients developed adenocarcinoma during a mean duration of follow-up of ~4 years (64). Eleven tumors were available for p53 immunostaining. Five of the 11 (45%) tumors stained positively for p53. Of the 11 patients that developed adenocarcinoma, biopsies at study entry were positive for p53 in 4 (36%) patients compared with 7 of 41 (17%) in the control cohort (P = 0.197). Although not statistically significant, this study may not have been adequately powered to detect a significant difference with the end point of adenocarcinoma. No results were provided regarding the ability of p53 to predict progression to the combined end point of high-grade dysplasia adenocarcinoma.

Reid et al. prospectively evaluated endoscopic biopsies in 269 patients for loss of heterozygosity (LOH) at the p53 locus, 17p, to determine if this loss increased the risk for neoplastic progression (65). Twenty of 54 (37%) patients with LOH at 17p progressed from metaplasia or dysplasia to esophageal adenocarcinoma during the study period compared with 6 of 202 (3%) patients in the patients without LOH (P < 0.001).

### Table 2. Studies of the relationship between p53 expression/mutation and neoplastic progression

<table>
<thead>
<tr>
<th>Study author year (ref.)</th>
<th>Histology</th>
<th>Total patients</th>
<th>Total p53-positive patients (%)</th>
<th>p53-positive patients with neoplastic progression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Younes 1993 (59)</td>
<td>No dysplasia/indefinite/low-grade dysplasia</td>
<td>24</td>
<td>3 (13)</td>
<td>2 (67)</td>
</tr>
<tr>
<td>Younes 1997 (60)</td>
<td>No dysplasia</td>
<td>36</td>
<td>0 (0)</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Gimenez 1999 (61)</td>
<td>Indefinite/low-grade dysplasia</td>
<td>25</td>
<td>9 (36)</td>
<td>5 (56)</td>
</tr>
<tr>
<td>Bani-Hani 2000 (64)</td>
<td>Not reported</td>
<td>52</td>
<td>11</td>
<td>4 (36)</td>
</tr>
<tr>
<td>Reid 2001 (65)</td>
<td>No dysplasia/indefinite/low-grade dysplasia</td>
<td>256</td>
<td>54 (21)</td>
<td>20 (37)</td>
</tr>
<tr>
<td>Weston 2001 (63)</td>
<td>No dysplasia/indefinite/low-grade dysplasia</td>
<td>197</td>
<td>19 (10)</td>
<td>5 (26)</td>
</tr>
<tr>
<td>Skacel 2002 (62)</td>
<td>Low-grade dysplasia</td>
<td>59</td>
<td>35 (59)</td>
<td>18 (51)</td>
</tr>
<tr>
<td>Dolan 2003 (66)</td>
<td>No dysplasia/low-grade dysplasia</td>
<td>48</td>
<td>10 (21)</td>
<td>3 (30)</td>
</tr>
<tr>
<td></td>
<td>High-grade dysplasia</td>
<td>16</td>
<td>9 (56)</td>
<td>7 (78)</td>
</tr>
<tr>
<td></td>
<td>Low-grade dysplasia</td>
<td>48</td>
<td>2 (4)</td>
<td>1 (50)</td>
</tr>
</tbody>
</table>
These investigators also found that the 5 of 19 (26%) patients with 17p LOH and biopsies showing no dysplasia or indefinite/low-grade dysplasia progressed to high-grade dysplasia/adenocarcinoma compared with 16 of 178 (9%) patients without 17p LOH (P = 0.02). Additionally, the patients with 17p LOH and high-grade dysplasia were more likely to progress to adenocarcinoma than patients with high-grade dysplasia and two normal p53 alleles (51% versus 21%, respectively; P = 0.03). This study provides convincing evidence that 17p LOH confers an increased risk for neoplastic progression. Of equal importance, however, was that 17p LOH was not necessary for neoplastic progression.

In another prospective surveillance program, DNA sequencing was used to identify p53 mutations and determine whether screening for these mutations could be of utility (66). Only 4% (2 of 48) of patients with metaplasia/low-grade dysplasia had detectable p53 mutations. Only 1 of these 2 patients with a p53 mutation progressed to adenocarcinoma. Importantly, in the 46 patients with wild-type p53, no patient progressed to adenocarcinoma. An important limitation of this study was that, even with small patient numbers, the presence of a p53 mutation was not specific for progression to malignancy (i.e., 1 patient with a p53 mutation did not progress to adenocarcinoma during the study follow-up of 4 years). Given the current cost of analyzing esophageal tissue for DNA mutations and the attendant morbidity of esophagectomy, the specificity and sensitivity of this test would likely need to be ~100% for this strategy to approach cost-effectiveness.

Barriers to Implementation

In addition to sensitivity and specificity of any biomarker test, direct and indirect costs are also major concerns with regards to their implementation into general clinical practice. Apart from the cost of performing the immunostaining, the determination of p53 positivity needs to be made by a pathologist, and this requires additional time and expense.

A critical component in the calculation of biomarker costs will be their effect on the frequency of surveillance endoscopy. It is clear from available studies that there are no currently accepted biomarkers that can identify with high-sensitivity patients who will progress to adenocarcinoma. In other words, no biomarker currently available would, if negative, merit discontinuation of screening. Additionally, no biomarker currently available is 100% specific for the development of adenocarcinoma (i.e., a biomarker that would have sufficiently high predictive power to recommend prophylactic esophagectomy). Additionally, in several studies, although the index biopsy was negative for p53 expression or dysplasia, subsequent biopsies were positive for p53 with low-grade dysplasia (59, 60, 62). Thus, a biopsy that is p53 negative does not obviate the need for further surveillance.

A recent cost analysis using a Markov model described the necessary criteria for a biomarker to be cost-effective in a surveillance strategy (5). In this model, a target population (Caucasian males with reflux disease) undergoes a screening endoscopy at age 50 years. Individuals without the presence of the indicator biomarker no longer undergo screening endoscopy (even in the presence of Barrett’s esophagus). Those who have a positive biomarker undergo surveillance endoscopy every 3 months until cancer is diagnosed. For biomarker-guided surveillance to be cost-effective, a biomarker would need to cost less than $100 and be 80% sensitive and specific. In a biomarker-guided esophagectomy strategy, patients with a positive biomarker at age 50 years undergo prophylactic esophagectomy. Using these criteria, a biomarker would need to have a 95% specificity rate for biomarker-guided esophagectomy to be cost-effective. Given the complex biology of carcinogenesis, it is unlikely a single biomarker could fulfill these criteria. A more optimistic approach would identify a panel of biomarkers that together might fulfill these criteria.

An additional impediment to implementation of biomarker-guided surveillance is that it will certainly not eliminate all adenocarcinomas unless upper endoscopy is routine rather than only based on symptoms of gastroesophageal reflux. In this regard, the absence of symptoms does not exclude Barrett’s esophagus and the potential need for surveillance. Rex et al. performed upper endoscopy on 961 patients who were scheduled for colonoscopy (68). Although long-segment Barrett’s esophagus was more common in those patients with heartburn symptoms, short-segment Barrett’s esophagus occurred with equal frequency in patients with or without reflux symptoms. Thus, although the presence of reflux may target an at-risk population, patients without reflux who would be excluded from screening may still harbor Barrett’s esophagus and go on to develop adenocarcinomas.

Finally, although p53 expression is a reasonable surrogate marker for many p53 gene mutations, it is still fairly inaccurate. It is estimated there is a 25% false-negative and 25% false-positive immunostaining results in comparison with direct sequencing for p53 mutations (36, 69). These errors cannot be further reduced by improvements in immunostaining techniques because they are the direct result of the biology of p53 rather than errors in testing. More accurate and cost-effective methods of detecting p53 mutations may eventually obviate the need for immunostaining.

**Table 2. Studies of the relationship between p53 expression/mutation and neoplastic progression (Cont’d)**

<table>
<thead>
<tr>
<th>Total p53-negative patients (%)</th>
<th>p53-negative patients with neoplastic progression (%)</th>
<th>p if reported</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 (88)</td>
<td>1 (5)</td>
<td>0.01</td>
<td>One patient with p53-positive biopsy lost to follow-up</td>
</tr>
<tr>
<td>36 (100)</td>
<td>0 (0)</td>
<td></td>
<td>Progression defined as high-grade dysplasia/adenocarcinoma in both groups</td>
</tr>
<tr>
<td>16 (64)</td>
<td>0 (0)</td>
<td></td>
<td>Some patients with two biopsies of differing histology</td>
</tr>
<tr>
<td>25 (100)</td>
<td>12 (48)</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>11 (65)</td>
<td>2 (18)</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>2 (33)</td>
<td>0 (0)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>0 (0)</td>
<td>Not applicable</td>
<td>0.197</td>
<td>Prospective cohort study; progression defined as adenocarcinoma</td>
</tr>
<tr>
<td>41</td>
<td>7 (17)</td>
<td>&lt;0.001</td>
<td>p53 positive defined as LOH; each row represents different subgroup analysis</td>
</tr>
<tr>
<td>202 (79)</td>
<td>6 (3)</td>
<td></td>
<td>Prospective enrollment</td>
</tr>
<tr>
<td>178 (90)</td>
<td>16 (9)</td>
<td>0.02</td>
<td>p53 positive defined as p53 mutation</td>
</tr>
<tr>
<td>24 (41)</td>
<td>5 (21)</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>38 (79)</td>
<td>2 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 (44)</td>
<td>1 (14)</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td>46 (96)</td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The biology of esophageal adenocarcinogenesis is complex. Given this complexity, it is probably naive to assume that a single biomarker might be able to discriminate those patients that will progress to high-grade dysplasia/adenocarcinoma. It is clear from the studies currently published that increased p53 expression as shown on immunostaining is associated with a higher risk of histologic progression. Patients who are p53 negative, however, may still go on to develop esophageal adenocarcinoma. With the available evidence, it would be irresponsible to recommend that p53 immunonegativity could be used to exclude patients from further screening. Given the noted interobserver variability in diagnosing dysplasia, immunohistochemical staining for p53 may be helpful in selected cases. With current evidence, it is reasonable to recommend more intensive screening in patients who are p53 positive. In the future, this may become irrelevant if a combination of biomarkers can be used to improve the sensitivity, specificity, and predictive power of p53 as a marker for malignancy.

Conclusions
