p53 Mutational Status Improves Estimation of Prognosis in Patients with Curatively Resected Adenocarcinoma in Barrett’s Esophagus

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ABSTRACT

The incidence of adenocarcinomas in Barrett’s esophagus has been rising in the last two decades in the United States and Western Europe for yet unknown reasons. We reported previously a large multi-institutional trial implicating p53 mutations as being involved in the pathogenesis of Barrett’s cancer and representing an early marker for the malignant potential of Barrett’s epithelium. A prospective study was performed to evaluate the prognostic impact of p53 mutations on survival in 59 patients with Barrett’s cancer. Tissue for DNA analysis was obtained by endoscopic biopsy or immediately after surgical resections from the tumor, Barrett’s epithelium, and normal stomach and esophagus. p53 mutation analysis was performed by PCR-single strand conformational polymorphism screening of exons 5–9 and DNA sequencing to unequivocally prove the presence of a mutation. p53 mutations were identified in 30 of 59 (50.8%) patients. The presence of a p53 mutation in the tumor had a significant impact on survival after curative resections (RO-resection) with cumulative 5-year survival probabilities of 68.8 ± 9.7% for mutation-negative tumors and 24.3 ± 9.9% for mutation-positive tumors (log rank: \( P < 0.001 \)). By Cox proportional hazard analysis, including the parameters of gender, age, Union International Contre Cancer tumor stage, grading, and p53 mutation status, only Union International Contre Cancer tumor stage (\( P < 0.0001 \)) and p53 mutation status (\( P < 0.02 \)) were of significant independent prognostic importance. p53 mutation analysis by DNA sequencing is of significant independent prognostic importance next to histopathological tumor stage in patients with curatively resected (RO-resection) Barrett’s cancer. It appears that p53 mutational status is a valuable parameter to define low-risk (p53 mutation-negative) and high-risk (p53 mutation-positive) groups for treatment failure after curative resections.

INTRODUCTION

Barrett’s esophagus develops as a complication in approximately 10–12% of patients with chronic gastroesophageal reflux disease (1) and is characterized by the replacement of the normal squamous epithelium of the esophagus with a metaplastic specialized columnar epithelium (2). The clinical importance of the detection of Barrett’s epithelium is attributable to its potential to undergo malignant transformation (3, 4). The incidence of Barrett’s cancer ranges from 1 in 55 to as low as 1 in 441 patient years in several prospective and retrospective studies (5) with an at least 30–40-fold increased risk for patients with Barrett’s esophagus (6). Interestingly, the incidence of adenocarcinomas in Barrett’s esophagus and gastric cardia increased more rapidly than any other cancer in the past two decades in the United States and Western Europe for yet unknown reasons (7, 8).

Severl studies support the concept that Barrett’s adenocarcinoma occurs as the result of tumor initiation and progression from benign metaplastic columnar epithelium to varying degrees of dysplasia, carcinoma in situ, and invasive carcinoma rather than a de novo tumor development (4, 9, 10).

Substantial evidence exists that Barrett’s cancer is associated with a clonal evolution process as a result of acquired genomic instability through a progressive accumulation of genetic abnormalities including the occurrence of single or multiple aneuploid cell populations (11–13). Allelic losses involving chromosome 17p and p53 protein overexpression were frequently detected in Barrett’s carcinomas and premalignant Barrett’s epithelium (14, 15).

Casson et al. (16) first demonstrated p53 mutations in Barrett’s cancer and premalignant Barrett’s epithelium, and the results were recently reconfirmed in a large multi-institutional prospective trial performed by our group (17). Controversial results were reported concerning the prognostic importance of p53 protein overexpression in Barrett’s cancer (18–20). One recent study with a heterogeneous patient population including squamous cell carcinomas, tumors of the gastric cardia, and adenocarcinomas in Barrett’s esophagus used SSCP5 analysis after PCR DNA amplification to

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5 The abbreviations used are: SSCP, single-strand conformational polymorphism; UICC, Union International Contre Cancer; NOD, negative for dysplasia; LGD, low-grade dysplasia; HGD, high-grade dysplasia.
Fig. 1 Representative SSCP analysis of exon 5 of the p53 gene. Five biopsies from a patient with Barrett’s cancer were analyzed: B1, peritumoral Barrett’s epithelium with high grade dysplasia; B2, Barrett’s epithelium with no evidence of dysplasia at the greatest distance to the tumor; T, tumor; E, normal squamous epithelium of the esophagus; G, normal epithelium from the gastric fundus. The tumor sample (T) shows an electromobility shift (arrow) indicative of a mutation that was subsequently proven by DNA sequencing.

define whether a p53 mutation was present and found no impact on survival (21).

The purpose of this prospective study was to evaluate the prognostic importance of mutations in exons 5–9 of the p53 gene identified by PCR-SSCP analysis and confirmed by DNA sequencing in patients with adenocarcinoma in Barrett’s esophagus.

MATERIALS AND METHODS

Study Design, Demographic Data, and Follow-up. A prospective study was performed in 59 patients with Barrett’s carcinoma recruited from the Departments of Surgery, Technische Universität München, Munich, Germany.

Barrett’s cancer was defined as an adenocarcinoma originating above the gastroesophageal junction in association with characteristic specialized columnar mucosa (22). The length of tumor-associated Barrett’s esophagus had to be ≥3 cm to include patients in this study. Exclusion criteria were a prior history of a malignant tumor or radiation and/or chemotherapy before sampling of the tissue specimens to be analyzed.

There were 54 (91.5%) male and 5 (8.5%) female patients, with a median age of 62.7 years (minimum, 41.7 years; maximum, 84.4 years). In 2 of 59 patients, Barrett’s cancer was detected during an endoscopic surveillance program for known Barrett’s esophagus; the remaining 57 patients were diagnosed with a malignant tumor in the esophagus that was subsequently classified as adenocarcinoma in Barrett’s esophagus.

Fifty-five of 59 (93.4%) patients received surgical resections, and the residual tumor category (R-category; Ref. 23) was distributed as follows: R0-resection, n = 49 (89.1%); R1-resection, n = 4 (7.3%); and R2-resection, n = 2 (3.6%). Resections were performed as radical transhiatal esophagectomy (24, 25) including partial proximal gastrectomy (proximal two-thirds of the lesser curvature to the uppermost tip of the gastric fundus). In addition to this partial lymphadenectomy of compartment I, complete lymphadenectomy in compartment II was performed (26).

Four of 59 (6.8%) patients were not resected and received definitive radiochemotherapy for palliation. Patients with R0-resections did not receive adjuvant chemotherapy and/or radiotherapy. In R1- or R2-resected tumors (n = 6), additive radiochemotherapy (5-fluorouracil; 64 Gy) was administered.

Staging was performed according to the UICC Tumor-Node-Metastasis Classification (27) and was distributed as follows: stage I, 22 (37.3%); stage II, 18 (30.5%); stage III, 14 (23.7%); and stage IV, 5 (8.5%). The histopathological grading of the primary tumor was classified as well-differentiated (G1) in 4 (6.8%), moderately differentiated (G2) in 19 (32.2%), and poorly differentiated (G3) in 36 (61%) patients.

Median follow-up was 44.1 months, and no patient was lost to follow-up. Patients were seen at 3-month intervals during the first postoperative year, every 6 months in the second and third years, and once a year thereafter. Evaluation consisted of physical examination; biochemical profile; chest radiograph; endoscopy; computed tomography of neck, chest, and abdomen; and abdominal ultrasound. Data on recurrences and cause of death were obtained for all patients.

Tissue Acquisition, Histology, and DNA Preparation. Tissue for DNA analysis was obtained by endoscopic biopsy or immediately after surgical resections from the following locations: tumor; peritumoral Barrett’s epithelium (BE-1); and Barrett’s epithelium taken from the greatest distance to the tumor (BE-2). If there was only a small area of macroscopic tumor-free Barrett’s epithelium (e.g., some pT2 or pT3 tumor categories), only peritumoral Barrett’s epithelium (BE-1) could be harvested and analyzed. Normal tissue for both groups were taken from the gastric fundus and squamous epithelium of the esophagus. All tissue specimens were immediately frozen in liquid nitrogen. Conventional histology for all tissue specimens analyzed was performed by a consultant gastrointestinal pathologist (K. B.).

The degree of dysplasia in metaplastic Barrett’s epithelium was evaluated according to the criteria of Riddell (28) and simplified to include only three categories as suggested by Williamson et al. (29): NOD, LGD, and HGD.

Peritumoral Barrett’s epithelium (BE-1) was analyzed in all patients, and the degree of dysplasia was determined as follows: NOD, 16 (28%); LGD, 23 (39%); and HGD, 19 (33%). A second specimen of Barrett’s epithelium taken from the greatest distance to the tumor (BE-2) could be analyzed in 26 of 59 patients and was classified as NOD in 10 (38.5%), LGD in 13 (50%), and HGD in 3 (11.5%) specimens.

This study was approved by the internal review board of the Technische Universität Muenchen, and informed consent was obtained from each patient.

DNA Extraction, PCR-SSCP Analysis, and DNA Sequencing. DNA preparation was carried out with a DNA extraction kit (Stratagene, Inc., La Jolla, CA). For all tissue sections taken at endoscopy or immediately after surgery, DNA extraction was performed from carefully selected sections from areas of Barrett’s epithelium (BE-1 or BE-2) or tumors as described (16). DNA analysis was performed for exons 5–9 for all specimens because >90% of mutations in the p53 gene occur in this evolutionary conserved part of the gene (30).

The protocol for PCR amplification, SSCP analysis, and DNA sequencing of the p53 gene, with a sensitivity to detect 1 mutated cell in 10 nonmutated cells, has been extensively described by us (17). SSCP analysis was carried out at least twice
for each specimen, and DNA sequencing was performed for all samples that showed an electromobility shift.

**Immunohistochemistry.** The polyclonal anti-p53 antibody CM-1 (Medac, Hamburg, Germany) was used for immunohistochemical detection of p53 protein in formalin-fixed, paraffin-embedded tissue sections from tumor-associated Barrett’s epithelium without evidence for dysplasia but presence of p53 mutations. The staining protocol has already been described extensively (31). As positive control, a Barrett’s cancer specimen with a known p53 transition-type mutation and p53 overexpression was chosen, and three specimens of tumor-associated Barrett’s epithelium without evidence of dysplasia and mutations in the p53 gene served as negative controls.

**Statistical Analysis.** Association of gene mutations with clinicopathological parameters were evaluated using the χ² test. Correlations between groups were assessed by Spearman’s correlation coefficient test. Survival was estimated according to Kaplan and Meier (32). Univariate analysis was performed with the log-rank test (33), multivariate analysis was performed with the Cox Proportional Hazard Regression Model (34), and significance was determined by χ² analysis. The level of significance was set to P < 0.05.

**RESULTS**

**Frequency, Location, and Type of p53 Mutations.** Mutations in the p53 gene were detected in 30 of 59 (50.8%) patients in tumor and/or associated Barrett’s epithelium. In 17 of 30 positive cases, the mutation was detected only in the tumor (type A), 9 of 30 showed mutations in tumor and Barrett’s

**Table 1** Location and type of p53 mutations in Barrett’s cancer and epithelium

<table>
<thead>
<tr>
<th>n (T)</th>
<th>n (BE)</th>
<th>Exon</th>
<th>Codon</th>
<th>Nucleotide change</th>
<th>Type</th>
<th>Amino acid change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>5</td>
<td>-splice site</td>
<td>G → A</td>
<td>TS</td>
<td>Cys → Tyr</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>5</td>
<td>173</td>
<td>CTG → TGT</td>
<td>TS</td>
<td>Leu → Cys</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>5</td>
<td>174</td>
<td>-1 bp (G)</td>
<td>DEL</td>
<td>Frameshift</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>5</td>
<td>175</td>
<td>CGC → CAC</td>
<td>TS</td>
<td>Arg → His</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>177-180</td>
<td>-11 bp</td>
<td>DEL</td>
<td>Frameshift</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>7</td>
<td>197</td>
<td>GTG → ATG</td>
<td>TS</td>
<td>Val → Met</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>7</td>
<td>237</td>
<td>-1 bp (G)</td>
<td>DEL</td>
<td>Frameshift</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>7</td>
<td>239</td>
<td>AAC → GAC</td>
<td>TS</td>
<td>Asn → Asp</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>7</td>
<td>248</td>
<td>CGG → CAG</td>
<td>TS</td>
<td>Arg → Gin</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>7</td>
<td>248</td>
<td>CGG → TGG</td>
<td>TS</td>
<td>Arg → Trp</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>7</td>
<td>249</td>
<td>-1 bp (G)</td>
<td>DEL</td>
<td>Frameshift</td>
</tr>
<tr>
<td>12</td>
<td>8</td>
<td>273</td>
<td>CGT → CAT</td>
<td>TS</td>
<td>Arg → His</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>8</td>
<td>275</td>
<td>TGT → TTC</td>
<td>TV</td>
<td>Cys → Phe</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>3</td>
<td>8</td>
<td>278</td>
<td>CCT → CTT</td>
<td>TS</td>
<td>Pro → Leu</td>
</tr>
<tr>
<td>15</td>
<td>2</td>
<td>8</td>
<td>282</td>
<td>CGG → TGG</td>
<td>TS</td>
<td>Arg → Trp</td>
</tr>
<tr>
<td>16</td>
<td>9</td>
<td>314</td>
<td>TCC → TCT</td>
<td>TS</td>
<td>Ser → Cys</td>
<td></td>
</tr>
</tbody>
</table>

a n (T), number of tumor samples; n (BE), number of samples from Barrett’s epithelium; Type, type of p53 mutation; TS, transition; TV, transversion; DEL, deletion.

**Table 2** Survival based on clinical parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>5-yr survival (%) ± SD</th>
<th>Median survival (mo)</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>54</td>
<td>39.7 ± 7.1</td>
<td>42.9 ± 12.3</td>
<td>18.7–67.2</td>
<td>0.91</td>
</tr>
<tr>
<td>F</td>
<td>5</td>
<td>50.0 ± 25.0</td>
<td>NR</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Age ≤ 60</td>
<td>18</td>
<td>25.0 ± 10.8</td>
<td>27.2 ± 15.4</td>
<td>0–57.4</td>
<td>0.39</td>
</tr>
<tr>
<td>≥ 60</td>
<td>41</td>
<td>43.8 ± 8.5</td>
<td>NR</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>UICC stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>22</td>
<td>79.3 ± 10.6</td>
<td>NR</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>II</td>
<td>18</td>
<td>41.9 ± 12.5</td>
<td>46.0 ± 18.5</td>
<td>9.7–82.4</td>
<td>0.0001</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>23.8 ± 13.1</td>
<td>25.2 ± 5.8</td>
<td>0–39.0</td>
<td>0.12</td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>0</td>
<td>1.6 ± 1.1</td>
<td>1.0–2.2</td>
<td>0.91</td>
</tr>
<tr>
<td>R-Category</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R0</td>
<td>49</td>
<td>48.7 ± 7.8</td>
<td>55.7</td>
<td>–</td>
<td>0.001</td>
</tr>
<tr>
<td>R1</td>
<td>4</td>
<td>0</td>
<td>25.2 ± 7.1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>R2</td>
<td>2</td>
<td>0</td>
<td>1.6</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>RX</td>
<td>4</td>
<td>0</td>
<td>1.4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Grading</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>4</td>
<td>75.0 ± 21.6</td>
<td>NR</td>
<td>–</td>
<td>0.75</td>
</tr>
<tr>
<td>G2</td>
<td>19</td>
<td>48.5 ± 11.9</td>
<td>55.7</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>G3</td>
<td>36</td>
<td>34.4 ± 8.6</td>
<td>26.3 ± 5.1</td>
<td>16.2–36.5</td>
<td>–</td>
</tr>
</tbody>
</table>

a CI, confidence interval; NR, not reached; –, not to be calculated; RX, definitive radiochemotherapy without resection.
epithelium with 7 of 30 concordant (type B) and 2 of 30 discordant mutations (type C). Four of 30 patients demonstrated p53 mutations only in Barrett’s epithelium (type D). A representative example (type A) is shown in Fig. 1.

Therefore, a total of 39 specimens (tumor, 26; BE-1, 11; and BE-2, 2) from 30 patients displayed p53 mutations, and the exon locations were distributed as follows: exon 5, 11; exon 6, 1; exon 7, 11; exon 8, 14; exon 9, 1; and splice site intron 4/exon 5, 1.

The predominant type of p53 mutations were transition-type missense mutations (n = 31; 79.5%), followed by deletion-type mutations (n = 7; 18%) and the rare transversion-type missense mutations (n = 1; 2.5%). The distribution of exon location and mutation type is summarized in Table 1.

Correlation of Clinicopathological Parameters with p53 Mutations and Protein Expression. The presence of p53 mutations did not correlate with the pT and pN categories, respectively, for UICC tumor stage and were present in 9 of 22 stage I, 7 of 18 stage II, 8 of 14 stage III, and 2 of 5 stage IV tumors. Nine of 23 pT1 tumors were positive for p53 mutations. There was no association of p53 mutations with the grading of the primary tumor. p53 mutations were present in 13 specimens of Barrett’s epithelium, and the degree of dysplasia was classified as NOD in 3, LGD in 3, and HGD in 7. Although p53 mutations occurred more frequently in HGD lesions, this association did not reach statistical significance. The type of p53 mutations in Barrett’s epithelium are shown in Table 1.

Immunohistochemical analysis for p53 protein expression in the three specimens without evidence for dysplasia and presence of p53 mutations showed that one sample was negative for p53 protein expression [exon 7, codon 249, 1bp (G) deletion], and two specimens were positive (exon 7, codon 248, CGG → CAG and exon 8, codon 278, CCT → CTT).

p53 Mutations and Survival. Cumulative 5-year survival probabilities based on several clinical parameters are shown in Table 2. In the following survival analysis, only patients with p53 mutations in the tumor (type A, B, and C mutations; n = 26) were classified as mutation positive. All other cases (n = 33) including type D mutations (n = 4) were considered mutation negative. The presence of p53 mutations in adenocarcinomas in Barrett’s esophagus was significantly associated with reduced survival by log rank testing (P < 0.006), and the 5-year cumulative survival probabilities were 55.1 ± 9.2% for mutation-negative and 20.9 ± 8.7% for mutation-positive cases.

For the 49 patients with complete resections (RO-resection), cumulative 5-year survival probabilities were 68.8 ± 9.7% for mutation-negative and 24.3 ± 9.9% for mutation-positive tumors, and statistical significance increased (P < 0.001). The respective survival curves are shown in Fig. 2. In a Cox proportional hazard analysis including the parameters age, gender, UICC tumor stage, grading, and p53 mutation, only UICC tumor stage (P < 0.0001) and p53 mutations (P < 0.02) were of significant independent prognostic importance. The significant parameters with their respective hazard ratios are shown in Table 3.

DISCUSSION

What clearly emerges from our analysis is that UICC tumor stage (P < 0.0001) and the p53 mutational status defined by DNA sequencing (P < 0.02) are independent prognostic factors for patients with adenocarcinoma in Barrett’s esophagus. Casson et al. (16) first reported on the presence of p53 mutations in Barrett’s epithelium and Barrett’s carcinomas in 14 adenocarcinomas of the esophagus including 7 unequivocally classified as Barrett’s cancer, and subsequently Neshat et al. (35) demonstrated p53 mutations in 7 of 12 patients with Barrett’s cancer. Our group recently reported the largest series of 96 patients including 48 patients with benign Barrett’s esophagus and 50 patients with Barrett’s cancer from four medical centers with p53 mutations present in 46% of patients with adenocarcinoma in Barrett’s esophagus (17). The frequency of p53 mutations (50.8%) of this analysis in 59 patients with Barrett’s cancer is consistent with earlier reports indicating a prevalence of p53 mutations in approximately 46–56% of patients with Barrett’s cancer (16, 17, 35). Gleeson et al. (36) reported p53 mutations in 69% of patients with Barrett’s cancer; however, only a total of 16 patients were examined, and reliable frequencies cannot be determined with such small sample sizes.

The presence of p53 mutations in the tumor (type A) or tumor and Barrett’s epithelium (type B) is consistent with a process of clonal evolution, a mechanism proposed by Nowell (37). Reid et al. (12) reported evidence using flow cytometry in a prospective study in patients with Barrett’s esophagus that progression to adenocarcinoma is associated with a clonal evolution process.

The occurrence of p53 mutation-negative tumors and mutation-positive Barrett’s epithelium (type D) could be explained with the field cancerization theory (38), implying that metaplastic Barrett’s epithelium of these patients would be at increased risk for cancer development. Multiple areas of various degrees of dysplasia can exist in the same patients with Barrett’s esophagus or Barrett’s cancer, including the occurrence of multiple aneuploid cell populations (12, 35), and progression to cancer in patients with Barrett’s esophagus has been shown to be associated with increased genomic instability (11). It is therefore likely that a p53 mutation-negative clone with a selective growth advantage formed a malignant tumor, and an area of
Barrett’s epithelium with an acquired p53 mutation was at an earlier step in the process of tumorigenesis. The same phenomenon also applies for discordant mutations between tumor and peritumoral Barrett’s epithelium (type C) as reported (17) and has also been observed in squamous cell carcinomas of the esophagus (39), suggesting that multifocal neoplasms can arise in both esophageal squamous cell carcinomas and Barrett’s associated adenocarcinomas.

To date only one study (21) has been reported that examined the prognostic importance of p53 mutations evaluated by DNA analysis in patients with Barrett’s cancer. Seventy-four esophageal carcinomas were examined; however, 46 were squamous cell carcinomas, 7 undifferentiated carcinomas, and only 21 were classified as adenocarcinomas arising in Barrett’s esophagus. Exclusively PCR-SSCP analysis without consecutive DNA sequencing was applied to determine the presence of a mutation. There was no significant impact of p53 mutations on survival by univariate and multivariate analysis; however, the data have to be interpreted carefully because various histological types of esophageal cancers were included in the study, and stage distribution was uneven because no patients with early stage (UICC stage I) cancer were included in the study. The heterogeneous patient population, including only a small number of patients with Barrett’s cancer (n = 21), therefore, does not allow any definitive conclusion on the prognostic impact of p53 mutations on survival.

Overexpression of p53 protein was already shown to be present in a subset of Barrett’s carcinomas (14). Ramel et al. (15) detected p53 protein overexpression in 8 of 15 (53%) patients with Barrett’s carcinoma. Younes et al. (40) found positive immunostaining for p53 in 87% of adenocarcinomas in Barrett’s esophagus. Rice et al. (41) reported that positive immunostaining occurred in 67% of patients with intramucosal cancer and 40% of submucosal cancer. A study by Jones et al. (42) showed significant p53 immunoreactivity in 70% of Barrett’s cancers.

From these studies, it is apparent that considerable variation in immunopositivity for p53 expression exists, and reported rates range between 40 and 87%. As a consequence, different results were reported concerning the impact of p53 protein overexpression on survival in esophageal adenocarcinomas. Casson et al. (18) prospectively studied 52 patients with esophageal adenocarcinomas and found immunopositivity in 28 of 52 (54%) tumors. p53 overexpression showed a trend toward reduced survival that was not statistically significant (P = 0.06, log-rank). Hardwick et al. (20) showed no significant impact on survival of p53 overexpression in 127 esophageal adenocarcinomas. Similarly, Duhamelgson and et al. (43) failed to find any association between p53 accumulation on survival in 42 patients with esophageal adenocarcinomas treated with neoadjuvant radiochemotherapy. Coggi et al. (21) examined 21 patients with Barrett’s cancer and did not see any prognostic importance of p53 expression.

On the contrary, Sauter et al. (19) showed, in a limited number of 24 patients with esophageal adenocarcinomas, significant improved survival for patients with tumors overexpressing p53 protein. The disparate results of the cited studies are difficult to interpret and could reflect observer variation in the interpretation, the use of different antibodies and staining procedures, as well as evaluation criteria for p53 immunopositivity.

Because p53 protein is expressed at very low levels in most cell types, increased wild-type p53 expression might be induced through nonmutational mechanisms (44). The correlation between increased immunoreactivity and the presence of mutations is imperfect. For example, frame-shift or chain-terminating (nonsense) mutations may not even be detected, because the resultant protein will be absent, truncated, or unstable (45).

From our previous work (17) and the presented analysis, false-negative results by p53 immunohistochemistry have to be expected in some 20–33% of patients because deletion-type mutations occur in that frequency.

p53 protein expression could, however, be potentially applied as an intermediate biomarker for the malignant potential of Barrett’s epithelium, as suggested by several authors (40–42). The fact that we could detect p53 mutations in Barrett’s epithelium without evidence of dysplasia strongly supports the hypothesis that p53 mutations occur early in the malignant degeneration of Barrett’s epithelium prior to cancer development. Because two of three samples showed p53 overexpression, this relatively simple technique could be easily applied. It appears, however, that up to one-third of patients might not be detected by applying p53 immunohistochemistry alone because deletion-type mutations might be falsely negative. Only a large prospective study in patients with Barrett’s esophagus can clarify this matter of discussion.

We further like to point out that the presence of an adenocarcinoma in the distal esophagus without histological proof of associated Barrett’s epithelium does not allow the unequivocal classification of the tumor as adenocarcinoma arising in Barrett’s esophagus. In esophageal adenocarcinomas without the presence of Barrett’s epithelium, the tumor could unequivocally be classified as adenocarcinoma arising in Barrett’s esophagus if the patient had endoscopy with histological proof of Barrett’s epithelium prior to tumor development. In the above-mentioned studies (18, 19, 20, 21, 43), adenocarcinomas in the distal esophagus with and without association of Barrett’s epithelium have been analyzed together. Recently, a trial has been reported demonstrating significant differences in survival for adenocarcinomas in the distal esophagus with and without the presence of Barrett’s epithelium (46), a fact that could sub-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Beta</th>
<th>SE</th>
<th>P</th>
<th>Hazard ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>UICC stage</td>
<td>0.926</td>
<td>0.549</td>
<td>0.001</td>
<td>2.52</td>
<td>0.86–7.40</td>
</tr>
<tr>
<td>Stage I/II</td>
<td>1.336</td>
<td>0.567</td>
<td>0.01</td>
<td>3.81</td>
<td>1.25–11.57</td>
</tr>
<tr>
<td>Stage I/III</td>
<td>2.840</td>
<td>0.676</td>
<td>0.0001</td>
<td>17.12</td>
<td>4.55–64.42</td>
</tr>
<tr>
<td>p53 mutation</td>
<td>0.902</td>
<td>0.394</td>
<td>0.02</td>
<td>2.47</td>
<td>1.13–5.34</td>
</tr>
</tbody>
</table>

*SE, standard error for beta; 95% CI, 95% confidence interval for hazard ratio.*
stentially contribute to variations of trial results. To account for this difference, we only included adenocarcinomas with histologically proven association to Barrett’s esophagus in our study.

The major conclusion of this study is that the presence of p53 mutations is an independent prognostic factor for patients with Barrett’s cancer in the subgroup of RO-resected tumors. The major advantage of this study is based on the fact that DNA sequencing was applied to unequivocally prove the presence of a p53 mutation and that exclusively patients with adenocarcinomas arising in histologically verified Barrett’s esophagus were included in the study.

It appears that the p53 mutational status is a valuable parameter to define low-risk (p53 mutation-negative) and high-risk (p53 mutation-positive) groups for treatment failure after curative resections. This information might be most valuable in the planning of pre- or postresection treatment strategies in future studies.

REFERENCES


30. Mantel, N. Evaluation of survival data and two new rank order statis

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p53 Mutational Status Improves Estimation of Prognosis in Patients with Curatively Resected Adenocarcinoma in Barrett's Esophagus

Paul M. Schneider, Oliver Stoeltzing, Jack A. Roth, et al.


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