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Clinical Significance of *p53* Mutations in Adenocarcinoma of the Esophagus and Cardia

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Objective

To compare the frequency and spectrum of *p53* gene mutations in adenocarcinomas of the esophagus and cardia and to compare clinical and pathologic features in patients with *p53* mutant and nonmutant cancers.

Summary Background Data

The *p53* gene is commonly mutated in human cancers, and a *p53* mutation is reported to be present in more than 50% of esophageal adenocarcinomas. Although many studies have investigated the frequency of p53 protein overexpression in adenocarcinomas of the esophagus or esophagogastric junction, few studies have assessed the frequency and clinical significance of *p53* mutations in these tumors. In particular, the prognostic importance of *p53* mutation is uncertain. Adenocarcinomas of the esophagus and cardia share many epidemiologic and pathologic features, but it is controversial whether they represent the same tumor. A comparison of the frequency and spectrum of mutations in adenocarcinomas of the esophagus and cardia would test whether these tumors are also similar at the molecular level.

Methods

DNA was isolated from microdissected paraffin-embedded tumor tissues of patients who underwent esophagogastrectomy for adenocarcinoma of the esophagus ($n = 19$), cardia (esophagogastric junction, $n = 12$), or subcardia ($n = 6$). Exons 5 to 8 of the *p53* gene were analyzed for the presence of mutations using the polymerase chain reaction with single-strand conformation polymorphism and DNA sequencing of bands showing abnormal mobility. The presence of mutation was confirmed by selective hybridization of a mutant-specific oligonucleotide to DNA isolated from the tumor.

Results

p53 mutations were identified in 18 of 37 (48.6%) tumors. Patients with *p53* mutant tumors were significantly younger and had a significantly poorer prognosis. There was a similar prevalence of *p53* mutations in adenocarcinomas of the esophagus (53%) and cardia (58%). In contrast, mutations were relatively uncommon in subcardia adenocarcinomas (one mutant tumor [17%]). The types of mutations found in the esophageal and the cardia cancers were also similar.

Conclusions

Adenocarcinomas of the esophagus and cardia have a similar frequency and spectrum of *p53* gene mutations, suggesting that these tumors have a common pathogenesis. Patients with mutations are younger, have signs of more advanced disease, and have a poorer prognosis than patients without mutations.

p53 gene abnormalities are common in human tumors,[1] and involvement of *p53*, by mutation,[2–10] altered mRNA expression,[11,12] allelic loss,[8,13–18] or protein overexpression, is found in a majority of esophageal adenocarcinomas. Although numerous studies have investigated the role of *p53* alterations in the development of esophageal adenocarcinoma and its precursor lesion Barrett esophagus, many of these studies measured immunohistochemical p53 protein overexpression as a surrogate for *p53* gene mutation. The reported accuracy of p53 protein expression measurement for the detection of mutation has varied widely,[3] but significant false-positive and false-negative rates have been found for immunohistochemistry compared with genomic mutation analysis in studies of many cancers,[1] including studies of esophageal adenocarcinoma.[9,19]

Despite the frequency and importance of *p53* alterations in the development of Barrett cancers, the clinical importance of these findings remains uncertain. In particular, whether there is an association between the *p53* mutation

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status of these cancers and the prognosis is uncertain. Studies that used immunohistochemical methods have reported varying results concerning p53 protein expression and clinical outcomes,[20–27] and few studies[9,28,29] have examined the prognostic value of *p53* mutation analysis in patients with esophageal adenocarcinoma. As a result, assessment of *p53* mutation, allelic loss, or p53 protein expression has not become part of the routine assessment of patients with Barrett esophagus or Barrett cancer.[30]

Molecular analysis of cancer tissues can be used to investigate the etiologic or other similarities of different tumor types. Adenocarcinomas of the esophagus and cardia (with the term *cardia* here indicating the esophagogastric junction and not the gastric cardia region in the proximal stomach) have some significant pathologic and epidemiologic similarities, but whether cardia cancers should be grouped with esophageal adenocarcinomas is disputed. This study was undertaken to investigate the genetic similarity of adenocarcinomas arising in the esophagus, cardia, and subcardia by determining the frequency and type of mutations in the *p53* gene in tumors from each site. A second aim of this study was to determine whether the presence of *p53* mutation in adenocarcinomas of the esophagus or cardia has any prognostic or other clinical importance for patients with these cancers.

PATIENTS AND METHODS

DNA was extracted from microdissected paraffin-embedded tumor tissue sections from 37 patients with adenocarcinoma of the esophagus, cardia, or subcardia. The location of the tumor was judged by examination of the resected esophagogastrectomy specimen. Tumors with an epicenter above the anatomical esophagogastric junction were classified as esophageal ($n = 19$), those with an epicenter at or within 1 cm of the esophagogastric junction were classified as cardia tumors ($n = 12$), and those with an epicenter 1 cm or more distal to the junction were classified as subcardia tumors ($n = 6$). Matching histologically normal squamous esophagus tissues were studied for each patient.

Exons 5 to 8 of the *p53* gene were amplified using the polymerase chain reaction (PCR). Amplified DNA from each tumor was compared with the other tumors and normal control DNA using single-strand conformation polymorphism (SSCP). The method of DNA isolation, primer sequences, PCR conditions, and method of autoradiography have been described previously.[31,32] PCR reactions were repeated on at least two occasions and SSCP gels were run under at least two different conditions: 6% acrylamide with and without 20% glycerol or Hydrolink (J.T. Baker, Phillipsberg, NJ) solution with or without 10% glycerol. Bands showing abnormal mobility on SSCP were excised and sequenced as previously described.[31,32] The sequence of the abnormal band was compared with the published wild-type *p53* sequence to identify mutations. To confirm the presence of mutation and to check nontumor tissue for the mutant sequence, 17 nucleotide long wild-type and mutant allele-specific oligonucleotides (ASO) were designed so that the mutant ASO matched the mutant sequence and the wild-type ASO matched the wild-type sequence over the same coding region. Oligonucleotides were end-labeled with $^{32}P\gamma ATP$ (0.1 mCi per microliter, NEN DuPont, Boston, MA), using T4 polynucleotide kinase (Boehringer Mannheim), and purified by separation on a 25G Sephadex column (Boehringer Mannheim), according to the manufacturer's instructions. Tumor and control DNA were amplified using the same conditions used for the SSCP that showed the abnormal band. The resulting amplified DNA was dot-blotted onto a nylon membrane using an automated laboratory workstation (Beckman Instruments 1000, Fullerton, CA). Forty microliters of each DNA sample was denatured with an equivalent volume of a solution containing 5 mol/L NaOH and 5 mmol/L EDTA. Half of each sample was then dripped onto separate halves of the membrane to produce two identical dots from each sample. Each dot was washed four times with 100 μL 20× SSPE, and the DNA was fixed to the membrane by ultraviolet irradiation (UV Stratalinker, Stratagene, La Jolla, CA). The membrane was divided and each half hybridized with either the wild-type or the mutant probe. Hybridization was carried out in 8 mL of a salt solution consisting of formamide 100 mL, Denhart's solution 50 mL, 20× SSC (Boehringer Mannheim) 100 mL, and dH_2O 225 mL, to which 5 to 10 μL of the ^{32}P-labeled probe was added. After hybridization, the membranes were washed in a salt solution consisting of 20× SSC 100 mL, 20% SDA (National Diagnostics) 5 mL, and dH_2O 900 mL. Hybridization and washing temperatures were tailored empirically to the probe being used. After washing, the membranes were placed in a cassette for autoradiography at $-70°C$ overnight. Mutations were confirmed by selective hybridization of the mutant ASO with the original tumor sample, without hybridization to normal DNA. The presence of amplified normal DNA was confirmed by hybridization of the wild-type ASO to the normal control sample.

The patients' notes were reviewed for clinical and pathologic details. An advanced tumor was defined as a tumor with transmural invasion, five or more lymph node metastases, or distant metastasis. The surgical procedure was classified as en bloc if the patient underwent resection of the tumor with extensive lymph node dissection with the intent of cure. If the patient did not undergo an en bloc resection, the surgical procedure was classified depending on the surgical approach as either transthoracic esophagectomy or transhiatal esophagectomy.

Statistical Analysis

The chi-square test was used to compare proportions between more than two groups, Fisher's exact test was used to compare proportions between individual groups, the Kruskal-Wallis test was used to compare continuous data between more than two groups, and the Mann-Whitney test was used to compare continuous data between individual groups. Survival curves were calculated using the Kaplan-Meier estimation method, and comparison between survival curves was with the log-rank test. $P < .05$ was accepted to denote statistical significance. Data are expressed as median with interquartile range (25th–75th percentiles) in parenthesis, unless indicated otherwise.

RESULTS

Comparison of Tumors by Location

The clinical characteristics of the patients, divided by location of tumor, are summarized in Table 1. Patients with tumors in the esophagus had a significantly longer history of heartburn than patients with tumors in the subcardia. Table 1 also shows the pathologic features of the tumors by location of tumor. Esophageal tumors were more likely to be associated with Barrett intestinal metaplasia than subcardia tumors ($P = .01$). Dysplastic Barrett epithelium was more frequently found adjacent to tumors located in the esophagus than tumors located in the cardia ($P = .003$). More distally located tumors tended to be more invasive, to have greater numbers of lymph node metastases, and to be less differentiated, but the small study population did not permit statistical analysis of these factors separately. However, if tumors with transmural invasion, five or more lymph node metastases, or distant metastasis were classified as advanced tumors (n = 27), then statistical analysis showed that advanced tumors were significantly less common in the esophagus than in the cardia or subcardia region.

p53 Mutations

p53 gene mutations were detected in 18 of the 37 tumors. Ten of the mutant tumors were esophageal cancers, seven were cardia cancers, and only one of the tumors in the subcardia region had a mutation. The epicenter of the mutant subcardia tumor was only 1 cm distal to the esophagogastric junction. The details of the mutations are summa-

Table 1. COMPARISON OF CLINICAL AND PATHOLOGIC FEATURES BY TUMOR LOCATION

Tumor location	Esophagus (n = 19)	Cardia (n = 12)	Subcardia (n = 6)	*P* Value
Clinical Features				
Male:female	17:2	11:1	4:2	NP
Age (years)	64 (52–70)	50 (47–60)	58 (55–74)	0.08
Heartburn				
Years	20 (3–30)	0 (0–17.5)	0.5 (0–1)	0.03
>5 years	13 (68%)	5 (42%)	0 (0%)	0.01
pack-years	30 (9–50)	11 (2–32)	15 (5–20)	0.29
≥20 pack-years	12 (63%)	5 (42%)	3 (50%)	0.49
Hiatal hernia	14 (87%)	4 (57%)	2 (40%)	0.07
Pathologic features				
Tumor size (cm)	4.5 (0.6–6.7)	3.9 (3.3–5.5)	4.8 (4–5)	0.09
Specialized epithelium	17 (89%)	7 (58%)	2 (33%)	0.02
Dysplasia	14 (74%)	2 (16%)	2 (50%)	0.006
Differentiation				
Well	4 (21%)	0 (0%)	0 (0%)	
Moderate	10 (53%)	3 (25%)	3 (50%)	
Poor	5 (26%)	9 (75%)	3 (50%)	NP
Depth of tumor invasion				
Intramucosal	3 (16%)	0 (0%)	0 (0%)	
Submucosal	2 (10%)	0 (0%)	0 (0%)	
Into wall	5 (26%)	2 (17%)	0 (0%)	
Through wall	9 (47%)	10 (83%)	6 (100%)	NP
Degree of nodal metastases				
None	6 (32%)	1 (8%)	0 (0%)	
1–4	6 (32%)	4 (33%)	3 (50%)	
≥5	7 (36%)	7 (58%)	3 (50%)	0.32
Presence of distant metastases	2 (11%)	0 (0%)	0 (0%)	NP
Advanced tumor	10 (53%)	11 (92%)	6 (100%)	0.01
p53 Mutation status	10 (53%)	7 (58%)	1 (17%)	0.22

NP, statistical analysis was not performed because of inadequate numbers.

rized in Table 2. An example of an autoradiogram showing an abnormal SSCP is shown in Figure 1, examples of mutant sequences are shown in Figure 2, and a representative dot-blot hybridization confirming the presence of mutation is shown in Figure 3.

No patient had more than one *p53* mutation detected. The mutant sequence was not detectable in normal nontumor tissue from any of the patients, indicating that the mutation was a somatic event. Most of the mutations were missense point mutations. There was one deletion of a single base pair that resulted in a frame shift. There were two insertions, one of three base pairs and the other of nine base pairs. In both cases the inserted sequence duplicated an immediately adjacent nucleotide sequence. Mutations were most commonly found in exons 5 and 7. Two patients had a mutation in codon 154, two in codon 175, and three codon 248 (see Table 2).

The most common mutations identified were transitions. G→A transitions were found in six tumors (three esophageal and three cardia); five of these were at CpG dinucleotides. A→G transitions were found in four patients (two esophageal and two cardia tumors). G→T transversions were less common, being found in four patients (two esophageal and two cardia). There was a marked similarity between the prevalence and mutation spectrum of tumors located in the esophagus and tumors located in the cardia.

Comparison of Clinicopathologic Factors in Patients With Mutant and Nonmutant Tumors

Patients with tumors in the esophagus and cardia were combined to permit examination of the relation between *p53* mutation and clinicopathologic features. The clinical features of patients with and without *p53* mutation are summarized in Table 3. Patients with *p53* gene mutations were 14.5 years younger on average than patients without *p53* mutations (Fig. 4, $P = .01$).

Patients with *p53* mutations tended to have more advanced tumors with respect to tumor size, differentiation, depth of invasion, degree of nodal metastasis, and presence of distant metastasis. However, there were no statistically significant differences in these factors. Curative resections with en bloc excision of the tumor and lymph nodes were performed in eight (47%) of the patients with mutation and in seven (50%) of the patients without mutation. Despite these nonsignificant differences in tumor stage and surgical treatment, patients with *p53* mutations had a significantly poorer prognosis than patients without *p53* mutations (Fig. 5). Two of the surviving four patients with *p53* mutations are disease-free, even though all were initially free of nodal disease. In contrast, five of the seven surviving patients without *p53* mutations are disease-free, even though three of the five initially had node metastasis. As expected from the data, patients with recurrent disease who had nonmutant

Table 2. LOCATION OF MUTATIONS IN p53 GENE, WITH NUCLEOTIDE AND AMINO ACID CHANGES

				Nucleotide Change		Amino Acid Change	
Patient ID	Location	Exon*	Codon	From	To	From	To
F1	Esophagus	5.5	135	T(G)C →	T(T)C	Phe →	Cys
F25	Esophagus	5.51	154	G(G)C →	G(T)C	Gly →	Val
F41	Esophagus	5.51	154	GGCO →	GGC(GGC)	Insert Gly	
F15	Cardia	5.51	161	(G)CC →	(A)CC	Ala →	Thr
F29	Esophagus	5.51	163	T(A)C →	T(G)C	Tyr →	Cys
F52	Subcardia	5.51	166	T0CA →	T(CACAGCACA)CA	Insert Gln His Thr	
F35	Cardia	5.51	172	G(T)T →	G(G)T	Val →	Gly
F23	Esophagus	5.51	175	C(G)C →	C(A)C	Arg →	His
F36	Cardia	5.51	175	C(G)C →	C(A)C	Arg →	Ilis
F26	Cardia	5.51	176	T(G)C →	T(T)C	Cys →	Phe
F30	Cardia	5.51	180	(G)AG →	(T)AG	Glu →	Stop
F5	Esophagus	6	215	(A)GT →	(G)GT	Ser →	Gly
F33	Cardia	7	239	(A)AC →	(G)AC	Asn →	Asp
F49	Esophagus	7	242	T(G)C →	TOC	Frameshift	
F50	Esophagus	7	248	(C)GG →	(T)GG	Arg →	Trp
F38	Cardia	7	248	C(G)G →	C(A)G	Arg →	Gln
F51	Esophagus	7	248	C(G)G →	C(A)G	Arg →	Gln
F53	Esophagus	7	249	(A)GG →	(G)GG	Arg →	Gly

* Exon 5.5 refers to the first half of exon 5; 5.51 refers to the second half of exon 5.

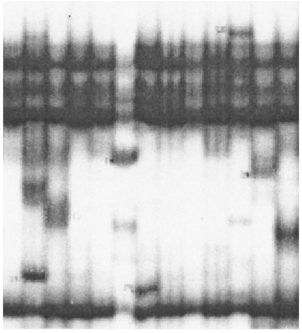

Figure 1. Autoradiogram from an single-strand conformation polymorphism gel of polymerase chain reaction products from exon 5 of the *p53* gene.

tumors had a better survival prognosis than patients with recurrent disease who had mutant tumors.

DISCUSSION

This study demonstrates that, in common with other tumors such as lung and colon cancers, approximately 50% of patients with adenocarcinoma of the esophagus or cardia (esophagogastric junction) have a *p53* mutation. *p53* mutations were detected in patients with all stages of tumor, including one patient who had a tumor confined to the lamina propria. This suggests that *p53* mutations occur early in the tumorigenic process, probably before the development of tumor. If this is so, then the presence of *p53* mutation may be a useful marker for identifying patients with Barrett esophagus who are at a greater risk of progression to adenocarcinoma. The results of several studies that showed an increased likelihood of disease progression in patients with Barrett esophagus with *p53* abnormalities support this possibility.[14,16,33,34] However, as shown in this study, not all patients with adenocarcinoma have a *p53* mutation, and the absence of *p53* mutation in patients with Barrett esophagus does not indicate that there is no risk of cancer development. Some authors have described *p53* mutations in nondysplastic Barrett metaplasia,[5,26,35,36] but the fate of patients with mutations in nondysplastic Barrett is unknown and will need to be assessed in prospective longitudinal studies. At present, the diagnosis of high-grade dysplasia on biopsy remains the best marker for the prediction of patients with occult cancer or those at highest risk of developing cancer.

The prevalence of *p53* mutations in adenocarcinomas of the esophagus and cardia in this study was higher than the mutation prevalence in patients with tumors of the subcardia region. These results are in keeping with those of Strickler et al,[31] who found *p53* mutations in 6 of 14 (43%) patients with tumors at the gastroesophageal junction compared with 2 of 26 (8%) patients with adenocarcinoma of the distal stomach ($P = .01$). The similar spectrum and prevalence of mutation in patients with tumors of the esophagus and cardia provide strong genetic support for the concept that these tumors have a common pathophysiology. Other similarities between esophagus and cardia cancers are a similar age at diagnosis,[37–40] a similar frequency of presentation at

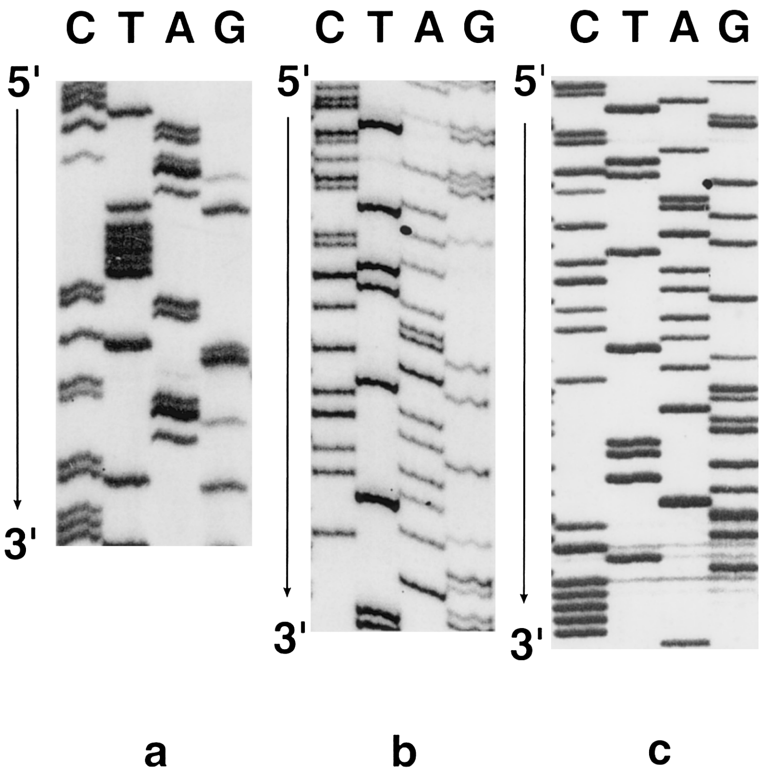

Figure 2. Sample autoradiograms of *p53* mutant nucleotide sequences. (A) Patient F1, codon 135, T(G)C→T(T)C. (B) Patient F15, codon 161, (G)CC→(A)CC. (C) Patient F29, codon 163, T(A)C→T(G)C.

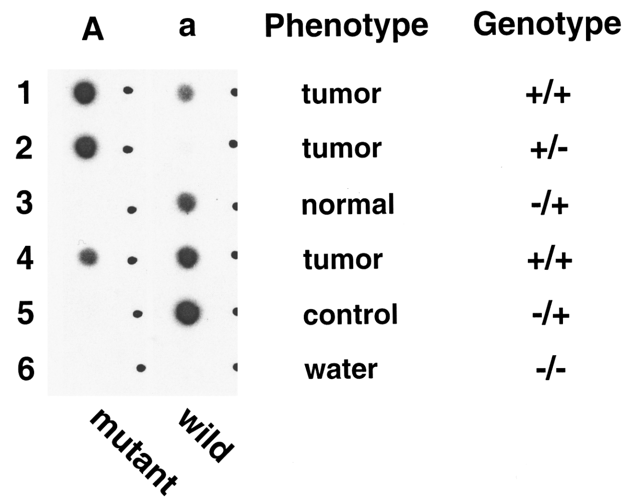

Figure 3. Autoradiogram of a dot-blot hybridization (patient F15) to confirm the presence of the mutation sequenced from an abnormal band seen on single-strand conformation polymorphism. DNA was isolated from tumor and normal tissues. Rows 1, 2, and 3 were obtained from microdissected tissue samples in an attempt to get as pure a tissue sample as possible. Row 4 was obtained from a larger dissection of the tumor and would contain more contamination of the tumor DNA with normal DNA from stromal elements. Row 4 acted as a positive control for the mutant because it was the source sample for the DNA used for the single-strand conformation polymorphism analysis. The DNA samples were amplified along with control normal DNA (positive control for wild-type DNA); then the polymerase chain reaction product was split and dripped onto two nylon membranes so that similar amplified DNA was present on each membrane. Membrane A was hybridized with the ^{32}P-labeled mutant allele-specific oligonucleotide (ASO) and membrane a with the labeled wild-type ASO. Specific hybridization of the mutant ASO to tumor sample (row 4) without hybridization of the mutant probe to the control DNA (row 5) shows that the mutant DNA sequence is present in the tumor sample but not in the control. Absence of the mutant DNA sequence in the patients' normal tissue (row 3) shows that the mutant DNA sequence is not present in the germ line. Row 2 shows that there is practically no wild-type DNA in the microdissected tumor sample, whereas strong hybridization of the mutant ASO confirms the presence of amplified DNA, in effect showing the "two hits" mutant DNA with loss of the wild-type allele.

late stages, and a similarly poor prognosis. Pathologic features of both tumors are similar, with similar gross morphologic features, and an association with the specialized intestinal metaplasia that characterizes Barrett esophagus has been shown in morphologic acid mucin staining studies.[37,37,38,38,40–42] Because of this, both esophageal and cardia cancers can be considered Barrett cancers.

Our group has also used the tissue and tumor specificity of retinoic acid receptor expression[43,44] to test the genetic similarity of esophagus and cardia adenocarcinomas. The retinoic acid receptor mRNA expression profile of these two tumors was similar,[45] and it was distinctly different from that reported for both noncardia gastric cancers[46–48] and esophageal squamous cell cancers.[47,49]

Further supporting evidence for a common pathophysiology comes from epidemiologic studies that report a rapid increase in the incidence of both esophageal and cardia adenocarcinomas during the same period that the incidence of noncardia gastric cancer was decreasing.[50–52] Epidemiologic surveys also show that in contrast to noncardia gastric cancers, both esophageal and cardia cancers are more common in white men.[37,41,50,51] An analysis of four studies[37,38,40,53] shows that the number of men for each woman with cardia adenocarcinoma varies from 2.3[39] to 11,[40] with an average male:female ratio of 5.5:1. The number of men for each woman with esophageal adenocarcinoma varies from 3.6[38] to 15,[37] with an average male:female ratio of 9.2:1. In tumors of the distal stomach, in contrast, there is an almost equal sex distribution (male:female ratio 1.1–1.3).[39,53]

Table 3. COMPARISON OF CLINICAL AND PATHOLOGIC FEATURES BETWEEN PATIENTS WITH AND WITHOUT p53 GENE MUTATIONS FOR PATIENTS WITH TUMORS IN ESOPHAGUS OR CARDIA

p53 gene	Mutated (n = 17)	Not Mutated (n = 14)	P Value
Clinical features			
Male:female	15:2	13:1	1.0
Age (years)	51 (49–62)	65.5 (61–70)	0.01
Heartburn			
Years	10 (0–20)	20 (0–30)	0.51
>5 years	10 (60%)	8 (57%)	1.0
Smoking			
Pack-years	15 (5–40)	25 (11–40)	0.69
≥20 pack-years	8 (47%)	9 (64%)	0.47
Hiatal hernia	10 (83%)	8 (73%)	0.64
Pathologic features			
Tumor size (cm)	5 (3–6.5)	3.9 (3.5–5)	0.47
Specialized epithelium	11 (65%)	13 (93%)	0.09
Dysplasia	6 (35%)	10 (71%)	0.07
Differentiation			
Well	2 (12%)	2 (14%)	
Moderate	6 (35%)	7 (50%)	
Poor	9 (53%)	5 (36%)	0.62
Depth of tumor invasion			
Intramucosal	1 (6%)	2 (14%)	
Submucosal	1 (6%)	1 (7%)	
Into wall	3 (17%)	4 (28%)	
Through wall	12 (71%)	7 (50%)	NP
Degree of nodal metastases			
None	3 (18%)	4 (28%)	
1–4	5 (29%)	5 (36%)	
>5	9 (53%)	5 (36%)	0.60
Presence of distant metastases*	2 (12%)	0 (0%)	0.49
Advanced tumor	13 (76%)	8 (57%)	0.44
Therapy (operation)			
Enbloc	8 (47%)	7 (50%)	
THE	6 (55%)	6 (43%)	
TTE	3 (18%)	1 (7%)	0.67

NP, statistical analysis was not performed because of inadequate numbers; THE, transhiatal esophagectomy, TTE, transthoracic esophagectomy.
* 2 patients had small metastatic lesions detected in the liver at the time of surgery.

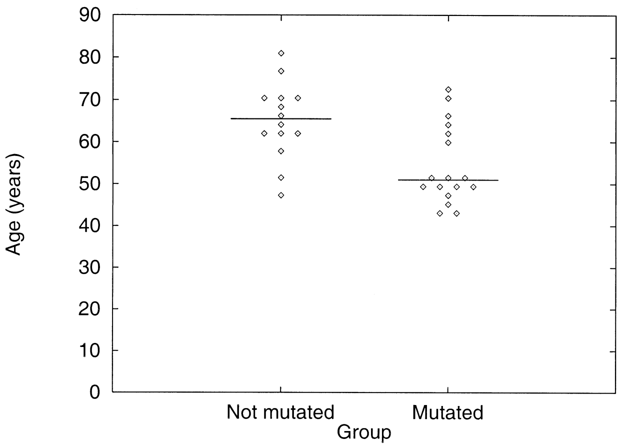

Figure 4. Relation between *p53* mutation status and age. Age of patients with *p53* mutant tumors is compared with that of patients with *p53* wild-type tumors. The horizontal bar represents the median value and each point represents the age of one patient. Patients with *p53* mutant tumors were significantly younger than patients with wild-type tumors.

Epidemiologic studies have also shown that risk factors for adenocarcinomas of the esophagus and cardia are similar, and that they are often significantly different from the known risk factors for noncardia cancers.[54–57] Chow et al[56] found that colonization with the *cag*+ genetic variant of *Helicobacter pylori* was significantly inversely associated with development of both esophageal and cardia adenocarcinomas. That study found no significant association between infection with this *H. pylori* subtype and development of noncardia gastric cancer, but other studies have found that *cag*+ *H. pylori* infection is significantly positively associated with development of gastric adenocarcinoma, including distal gastric cancers.[58–60] An inverse association between *H. pylori* infection and the presence of Barrett esophagus has also been found.[61–64] It is thus possible that certain types of *H. pylori* infection may promote noncardia gastric adenocarcinoma development but may protect against the development of Barrett esophagus and the Barrett-associated adenocarcinomas of the esophagus and cardia.

The mutation spectrum for adenocarcinoma of the esophagus or cardia can be examined further by combining the 17 mutations in the present study with the 36 reported in the literature.[2,3,5,11,36] There is a high prevalence of transition mutations—that is, replacement of the purine adenine (A) with the purine guanidine (G) or vice versa, or of the pyrimidine thymidine (T) with the pyrimidine cytosine (C) or vice versa. Overall, 38 (73%) of the 53 reported mutations are transitions either from G→A or from A→G. This type of mutation is commonly believed to be the effect of an external carcinogen.[1] In particular, G→A transitions may be due to the formation of O^6 guanidine DNA adducts by methylating nitrosamines.[65–68] Indeed, 30/38 (79%) of these transitions are G→A, which indicates that a G:C base pair has changed to an A:T base pairing. The change could be due to either G→A on one DNA strand or from C→T on the opposite strand. The main cause of C→T transitions is thought to be "spontaneous" deamination of 5-methyl cytosine to thymine.[69–72] In a recent review, 451 of 960 (47%) *p53* mutations in colon cancer were C→T transitions at CpG dinucleotides.[1] This prevalence is similar to the 49% prevalence of this type of mutation in esophageal adenocarcinoma, indicating that the spectrum of mutations in esophageal adenocarcinoma is similar to that in adenocarcinoma of the colon.

A significant finding in our study was that patients with *p53* mutations were younger than those without a *p53* mutation (see Fig. 4). This age difference may indicate that the *p53* route to tumorigenesis represents a shortcut, in that patients with a *p53* mutation progressed to invasive tumors 15 years on average before patients without a *p53* mutation. This clinical association has not been previously described for adenocarcinoma of the esophagus or cardia, but Zheng et al[73] reported that patients with squamous cancer of the lung in whom a *p53* mutation was detected were 11 years younger on average than patients in whom mutation was not detected (54 vs. 65 years, $P < .001$).

In the present study, in addition to being younger, patients with *p53* mutations tended to have more advanced tumors (see Table 3), and the prognosis was significantly worse in patients with *p53* mutant tumors than in those with wild-type tumors (see Fig. 5). Reduced survival in patients with esophageal adenocarcinoma with *p53* alterations has also been reported in two other recent studies that included DNA sequencing analysis.[28,29] If the results of these studies and the present study are consistently observed in other large studies, these findings will indicate that "molecular staging" of these tumors by analysis of the *p53* gene (and other genes that significantly distinguish between different Barrett stages[45,74–78]) may be worthwhile. However, at

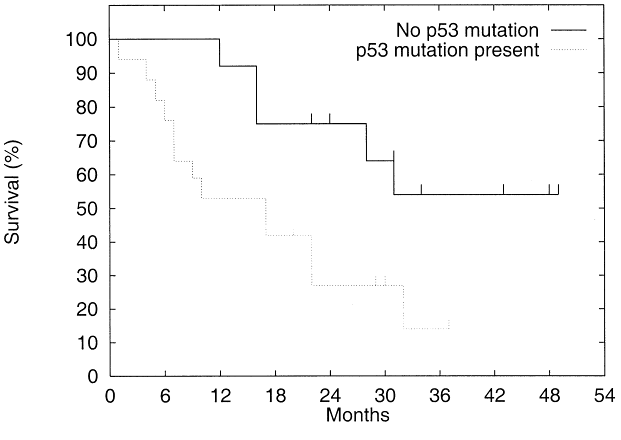

Figure 5. Effect of *p53* mutation status on survival. Actuarial survival of patients with *p53* mutations is compared with that of patients without *p53* mutations. Patients with subcardia tumors and patients who died of postoperative complications are excluded. Patients with mutant tumors had significantly worse survival than patients with wild-type tumors. Censored values are denoted by a tick.

present the best prognostic indicator for patients with these cancers remains tumor stage.

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