Genetic Alterations in Barrett Esophagus and Adenocarcinomas of the Esophagus and Esophagogastric Junction Region

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The incidence of esophageal adenocarcinoma has increased markedly in the past two decades, but the genetic alterations in this cancer and its precursor, Barrett mucosa, have not been characterized extensively. DNA replication errors and allelic losses of chromosomes 17p, 18q, and 5q were studied in 36 resected adenocarcinomas arising in the esophagus and esophagogastric junction, 56 Barrett adenocarcinomas, and 11 dysplasias in Barrett esophagus. The results were compared with clinical and pathological characteristics, including patient survival. Replication error positive cancer was rare (5.4%) in esophageal adenocarcinomas and was not found in Barrett mucosa. There was an increase in the prevalence of chromosomal losses in the Barrett mucosa–columnar dysplasia–adenocarcinoma sequence: 17p loss occurred in 14% of Barrett mucosae, 42% of low-grade dysplasias, 79% of high-grade dysplasias, and 75% of adenocarcinomas, respectively; loss of 18q in 32%, 42%, 73%, and 69%; and loss of 5q in 10%, 21%, 27%, and 46%. Clinical stage was a very strong prognostic factor for survival, and adenocarcinomas with allelic loss of both 17p and 18q had worse survival than cancers with no or one allelic loss ($P = 0.002$). Our results indicate that accumulation of genetic alterations follows the dysplasia–adenocarcinoma sequence in the esophagus and that losses of 18q and 17p occur earlier than 5q loss. Allelic loss of both 17p and 18q in esophageal adenocarcinoma identifies patients with poor prognosis. (*Am J Pathol* 1998, 153:287–294)

The incidence of adenocarcinoma of the esophagus and esophagogastric junction (EGJ) region has increased markedly in the United States in the past 20 years.^{1,2} The

etiology and pathogenesis of esophageal and EGJ adenocarcinomas remain unclear, but Barrett esophagus characterized by columnar epithelial metaplasia due to chronic injury from gastroesophageal reflux is an identified predisposing condition.^{3,4} Columnar epithelial dysplasia in Barrett mucosa is now regarded as a premalignant lesion.^{5,6}

Studies of genetic abnormalities in esophageal adenocarcinoma and its precursor have shown alterations in the *p53* tumor suppressor gene, an important regulatory gene in cell cycle control and apoptosis, and the *p53*-regulated cyclin-dependent kinase inhibitor *p21^{WAF1/CIP1}*.^{7–14} Allelic losses in multiple chromosomal loci of known tumor suppressor genes have also been reported.^{15–19} These include chromosome 17p (*p53* gene), 18q (*DCC*, *DPC4*, and *JV18-1*), 5q (*APC*), and 9p (*p16*). In contrast to colorectal adenocarcinomas, allelic losses in esophageal adenocarcinomas are rarely associated with mutations in the corresponding tumor suppressor genes except for 17p allelic loss and *p53* gene mutation.^{20–22} DNA replication errors (RERs) manifested as microsatellite instability due to defective repair of nucleotide mismatches have been reported infrequently in esophageal adenocarcinomas.^{23–25}

Clinical associations with these molecular genetic alterations have not been investigated extensively. Overexpression of *p53* suppressor gene product and expression of *p21^{WAF1/CIP1}* were found to have no prognostic value in patients with surgically resected esophageal adenocarcinomas in our previous study.¹⁰ The roles of RER status and multiple chromosomal allelic losses in the development of esophageal adenocarcinomas remain unclear, and their prognostic implications have not been studied. We therefore evaluated RER; loss of 17p, 18q, and 5q; and overexpression of *p53* gene product in resected adenocarcinomas of the distal esophagus and EGJ region and compared the findings with clinical and pathological features, including patient survival.

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Materials and Methods

Patient Selection and Follow-up

Ninety-two patients (82 men and 10 women) with adenocarcinoma of the distal esophagus or EGJ region in an esophagectomy specimen and 11 patients (9 men and 2 women) with Barrett esophagus and columnar epithelial dysplasia in a prophylactic esophagectomy specimen were identified from the computerized surgical pathology files of The Johns Hopkins Hospital from 1988 to 1996 and had specimens available for study. Among the 92 patients with adenocarcinoma, 56 (61%) had Barrett mucosa, 11 (12%) had undergone preoperative chemotherapy or irradiation, and 24 (26%) were treated with postoperative chemotherapy or irradiation. Follow-up information was obtained from The Johns Hopkins Hospital Tumor Registry and chart review.

Pathological Evaluation

Routine hematoxylin and eosin-stained slides from the resection specimens were evaluated for the presence of Barrett mucosa, columnar epithelial dysplasia (low-grade or high-grade),³ grade of tumor differentiation (well, moderate, and poor), depth of tumor invasion, and lymph node metastasis.²⁶ The nondysplastic Barrett mucosa included Barrett mucosa negative for dysplasia and indefinite for dysplasia. Paraffin blocks of adenocarcinoma ($n = 92$), Barrett mucosa with high-grade dysplasia (HGD; $n = 33$, including 24 accompanied by adenocarcinoma and 9 from prophylactic esophagectomy specimens), Barrett mucosa with low-grade dysplasia (LGD; $n = 19$, including 14 accompanied by adenocarcinoma and 5 from prophylactic esophagectomy specimens), nondysplastic Barrett mucosa ($n = 22$, including 19 accompanied by adenocarcinoma and 3 from prophylactic esophagectomy specimens), and squamous-lined esophageal mucosa ($n = 92$) were selected for immunohistochemical study and microdissection for DNA extraction. In 51 of 56 adenocarcinomas with Barrett esophagus, specimens of Barrett mucosa were satisfactory for immunohistochemistry and allelic loss analysis; 24 (47%) had HGD, 11 (22%) had LGD, and 16 (31%) were negative or indefinite for dysplasia.

The adenocarcinomas were staged according to the Union Internationale Contre le Cancer TNM system.²⁷ Tumors arising at the EGJ without evidence of Barrett mucosa were staged as esophageal cancer if more than 50% of the tumor was above the EGJ and as gastric cancer if more than 50% of the tumor was in the stomach.²⁸

RER and Allelic Loss of Chromosomes 17p, 18q, and 5q

Histopathological sections from H&E-stained slides were microdissected for DNA extraction. Genomic DNA was extracted as described previously.²⁹ Loss of heterozygosity (LOH) on the short arm of chromosome 17 (17p) was assessed by microsatellite assays using polymerase

Figure 1. Allelic loss of chromosome 17p in the region of the *p53* tumor suppressor gene in adenocarcinoma (Ca) and high-grade columnar epithelial dysplasia (H) as compared with the control nonneoplastic esophageal mucosa (N) of the same patient. Allelic loss (arrow) is evidenced by the absence of one heterozygous band (D17S520) and a 50% decrease in intensity in two others (VNTR and D17S1176).

chain amplifications of three microsatellite markers (TP53, D17S520, and D17S1176) and one tandem sequence repeat marker (VNTR, Figure 1), LOH on the long arm of chromosome 18 (18q) by eight microsatellite markers (D18S57, D18S65, D18S69, D18S64, D18S55, D18S61, D18S58, and D18S70, Figure 2), and LOH on the long arm of chromosome 5 (5q) by three microsatellite markers (D5S299, D5S82 and D5S346). In brief, polymerase chain reaction-based microsatellite assays were performed in 96-well plates with 38 cycles (95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute) using PCR Master (Boehringer Mannheim, Mannheim, Germany) in a 10- μ l volume and 10 ng of both 5' and 3' oligonucleotides. The 5' oligonucleotide was end labeled with [γ^{32} P]ATP (NEN DuPont, Boston, MA) using T4 polynucleotide kinase (New England Biolabs, Beverly, MA). The polymerase chain reaction products were analyzed on 6% polyacrylamide gel containing 7 mol/L urea.

Allelic shift (instability) of a microsatellite was defined by the presence of at least one additional band in the DNA from the tumor or Barrett mucosa that was not present in the control nonneoplastic sample (Figure 3). A specimen was considered as RER+ when allelic shifts were present in at least 4 of the 14 dinucleotide microsatellite markers.

Allelic loss of a marker was considered to be present when the microsatellite demonstrated absence or at least 50% decrease in intensity of a heterozygous band from the tumor or Barrett mucosa samples as compared with the control esophageal mucosa. For chromosome 17, loss of the short arm was considered to be present when more than two evaluable microsatellite markers showed allelic loss. For chromosomes 18 and 5, loss of the long arm was considered as complete when all of the evalu-

Figure 2. Allelic loss of chromosome 18q in adenocarcinoma (Ca) and high-grade columnar epithelial dysplasia (H) as compared with control nonneoplastic esophageal mucosa (N) of the same patient. The adenocarcinoma showed complete loss of 18q (arrows), whereas the HGD had partial loss (D18S55, D18S61, and D18S58).

able microsatellite markers showed allelic loss, and as partial when only the microsatellite markers close to the telomere showed loss. For statistical analysis, any tumor or Barrett mucosa with complete or partial chromosomal loss of 18q and 5q was considered as positive for allelic loss.

Immunohistochemistry for p53 Gene Product

Immunoperoxidase staining using diaminobenzidine as chromogen was performed on histopathological sections from formalin-fixed paraffin-embedded tissue. p53 tumor suppressor gene product (mouse monoclonal antibody DO7 (DAKO, Nutley, NJ) at a dilution of 1:100) was stained in deparaffinized sections after antigen retrieval using a heat-induced epitope retrieval method.³⁰ The TechMate 1000 automatic staining system (BioTek Solutions, Tucson, AZ) was used. Percentage of nuclei with staining in adenocarcinomas and Barrett mucosa was

estimated. p53 overexpression was defined by staining of more than 50% of the nuclei.³¹ The results of p53 immunohistochemistry for 76 of the patients with adenocarcinoma and 6 of the patients with Barrett mucosa were published previously.¹⁰

Statistical Analysis

Associations of molecular markers with clinical and pathological features were evaluated by Fisher's exact test. Factors tested for prognostic value included the presence of Barrett mucosa; stage of disease; preoperative or postoperative chemoradiation; grade of tumor differentiation; overexpression of p53 tumor suppressor gene product; and allelic loss of 17p, 18q, and 5q. The major statistical endpoint of survival analysis was the duration of survival after surgical resection of tumor. The event-time distributions for this endpoint were estimated by the method of Kaplan and Meier³² and compared using log-rank statistics³³ or the proportional hazards regression model.³⁴ The simultaneous effect of two or more factors was studied using the Cox multivariate proportional hazards model. Covariates marginally significant ($P < 0.19$) in the univariate analysis were entered into the multivariate regression model, and nonsignificant effects were removed in a stepwise fashion. All P values reported were two sided. Computations were performed using the Statistical Analysis System (SAS Institute Inc, Cary, NC) or EGRET (Statistics and Epidemiological Research Corp., Seattle, WA).

Results

Clinical and Pathological Features

Among the 92 adenocarcinomas, 56 (61%) were accompanied by Barrett mucosa. There was a male predominance in patients who had adenocarcinoma with and without Barrett mucosa (88% and 92%, respectively) and in patients with prophylactic esophagectomy for dysplasia in Barrett esophagus (82%). The demographic and pathological findings are summarized in Table 1. There were no significant differences in the grade of differenti-

Figure 3. RER in adenocarcinoma (Ca) with nondysplastic Barrett mucosa (B). Microsatellite instability in the adenocarcinoma is evidenced by the presence of additional bands (arrows) in the three microsatellite markers shown, but the Barrett mucosa is RER-.

Table 1. Clinical and Pathological Features of Patients

	% male (n)	Mean age ($\pm$ SD)	Tumor differentiation			Stage			
			% well (n)	% moderate (n)	% poor (n)	% I (n)	% II (n)	% III (n)	% IV (n)
Adenocarcinoma without Barrett (n = 36)	92 (33)	58.5 $\pm$ 9.5	0 (0)	81 (29)	19 (7)	0 (0)	22 (8)	56 (20)	22 (8)
Adenocarcinoma with Barrett (n = 56)	88 (49)	65.5 $\pm$ 6.9	2 (1)	73 (41)	25 (14)	20 (11)	29 (16)	39 (22)	12 (7)
All Adenocarcinoma (n = 92)	89 (82)	63.0 $\pm$ 8.8	1 (1)	76 (70)	23 (21)	12 (11)	26 (24)	46 (42)	16 (15)
Prophylactic esophagectomy									
HGD (n = 9)	78 (7)	56.0 $\pm$ 7.8							
LGD (n = 2)	100 (2)	52.5 $\pm$ 2.5							

ation or pathological stage between adenocarcinomas with and without Barrett mucosa.

RER Status

RER was rare, present in only 3.6% (2 of 56) of adenocarcinomas with Barrett mucosa (Figure 3) and 8.3% (3 of 36) of adenocarcinomas without Barrett mucosa. The number of markers shifted ranged from 4 of 14 to 13 of 14. Histopathologically, 4 RER+ adenocarcinomas showed poor differentiation, and the other one had moderate differentiation. Among the 4 poorly differentiated adenocarcinomas, 3 had features of medullary carcinoma with lymphoid infiltrate.³⁵

No RER was seen in any HGD, LGD, or nondysplastic Barrett mucosa; in the two RER+ adenocarcinomas with Barrett mucosa, allelic shift was seen in only 1 of 14 microsatellite markers in the accompanying nondysplastic Barrett mucosa as contrasted with 4 of 14 and 9 of 14 in the adenocarcinomas.

Allelic Loss of Chromosomes 17p, 18q, and 5q

As shown in Figure 4, accumulation of chromosomal losses occurred in the neoplastic sequence from nondys-

Figure 4. Accumulation of genetic alterations in the morphological dysplasia–adenocarcinoma sequence in the distal esophagus and EGJ region. B, non-dysplastic Barrett mucosa; LGD, low-grade columnar epithelial dysplasia; HGD, high-grade columnar epithelial dysplasia; CA-B, adenocarcinoma with Barrett mucosa; CA, adenocarcinoma without Barrett mucosa; IHC, immunohistochemical stain; RER+, DNA replication errors. All alterations were more common in adenocarcinoma and HGD than in nondysplastic Barrett mucosa.

plastic Barrett mucosa to LGD, HGD, and adenocarcinoma; loss of 17p as compared with squamous-lined mucosa was present in 14% of Barrett mucosa, 42% of LGD, 79% of HGD, and 75% of adenocarcinomas, respectively ($P < 0.00001$ for adenocarcinoma *versus* Barrett mucosa, Figure 1); loss of 18q in 32%, 42%, 73%, and 69%, respectively ($P = 0.003$ for adenocarcinoma *versus* Barrett mucosa, Figure 2); and loss of 5q in 10%, 21%, 27%, and 46%, respectively ($P = 0.001$ for adenocarcinoma *versus* Barrett mucosa). The majority (64%) of nondysplastic Barrett mucosa had no allelic loss.

Accumulation of alterations was also evident in paired specimens of adenocarcinoma and Barrett mucosa from individual patients. When allelic loss of 17p, 18q, or 5q was identified in an adenocarcinoma, the associated Barrett mucosa had the same losses in 59% (22 of 37), 62% (21 of 34), and 27% (6 of 22) of the patients, respectively. Allelic loss was present in dysplastic Barrett mucosa but not in associated adenocarcinoma in only 2 cases for 17p, 2 cases for 18q, and 1 case for 5q.

Multiple chromosomal allelic losses of 17p, 18q, and 5q were seen in 30% of adenocarcinomas either with or without Barrett mucosa, but rarely in nondysplastic Barrett mucosa (4%, $P = 0.02$; Figure 5). The allelic losses of chromosomes 17p, 18q, and 5q in cancers were not associated with clinical stage, tumor differentiation, patient age, or presence of Barrett mucosa. HGD of prophylactic esophagectomy specimens and stage I adenocarcinomas had no significant differences in frequency of 17p loss (78%, 7 of 9, and 64%, 7 of 11, respectively), 18q loss (89%, 8 of 9, and 55%, 6 of 11, respectively), and 5q loss (44%, 4 of 9, and 18%, 2 of 11, respectively).

p53 Immunohistochemistry

Overexpression of p53 gene product was seen more frequently in adenocarcinomas and HGD (54% and 71%, respectively) than in LGD (31%) and nondysplastic Barrett mucosa (5%) ($P = 0.00007$ for adenocarcinoma *versus* Barrett mucosa, Figure 4). Overexpression of p53 gene product was not seen in nonneoplastic squamous-lined mucosa. Furthermore, there were no significant differences in frequency of p53 overexpression between

Figure 5. Multiple allelic losses of chromosomes 17p, 18q, and 5q. Multiple losses were common in high-grade columnar epithelial dysplasia (HGD), adenocarcinomas with Barrett mucosa (CA-B), and adenocarcinomas without Barrett mucosa (CA), but less frequent in low-grade columnar epithelial dysplasia (LGD) and nondysplastic Barrett mucosa (B).

HGD in prophylactic esophagectomy specimens and stage I adenocarcinomas (67% and 55%, respectively). Similarly, there were no significant differences in p53 overexpression between adenocarcinomas with or without Barrett mucosa (49% and 58%, respectively). Overexpression of p53 gene product was present in 60% (3 of 5) of RER+ esophageal adenocarcinomas.

p53 Overexpression and 17p Allelic Loss

p53 overexpression and 17p allelic loss were concordant in 63% (57 of 90) of adenocarcinomas and in 71% (49 of 69) of Barrett mucosa with or without dysplasia. p53 overexpression was seen in 64% (42 of 66) of adenocarcinomas with 17p allelic loss but in 37% (9 of 24) of adenocarcinomas without 17p allelic loss ($P = 0.048$); it was also seen in 62% (21 of 34) of Barrett mucosa with 17p allelic loss but in 20% (7 of 35) of Barrett mucosa without 17p allelic loss ($P = 0.0006$).

Prognosis of Adenocarcinomas

The overall median survival for patients with resected adenocarcinomas in the distal esophagus and EGJ region was 16.3 months. The Kaplan-Meier survival curves by stage are shown in Figure 6. Clinical stage of disease was a very strong prognostic factor for survival (Table 2). The median survival times for stages I–IV were 66, 22, 11, and 9 months, respectively, and the hazards ratios (HRs) for stages II–IV as compared with stage I were 7.6 ($P = 0.007$), 12.2 ($P < 0.001$), and 20.4 ($P < 0.001$).

RER+ cancers showed a trend toward improved survival as compared with RER– tumors. The median survival time for five patients with RER+ tumors (3 with stage II and 2 with stage III) was 35 months as contrasted with 22 months in stage II and 11 months in stage III, although the differences were not statistically significant.

Allelic loss of chromosome 18q was a significant prognostic indicator (HR = 1.8, $P = 0.04$), and allelic loss of 17p (HR = 1.8, $P = 0.06$) and 5q (HR = 1.5, $P = 0.08$) was marginally associated with survival in a Cox proportional hazards model. As shown in Table 2, preoperative or postoperative chemoradiation, presence of Barrett mucosa, and p53 overexpression were not prognostic indicators. When the allelic losses of 17p, 18q, and 5q were adjusted for stage of disease in a multivariate regression model, only allelic loss of 17p (HR = 1.8, $P = 0.06$) and 18q (HR = 1.7, $P = 0.08$) remained marginally predictive of survival (Table 3). Statistically significantly worse survival was noted in patients with adenocarcinomas that had allelic losses of both 17p and 18q as compared with no allelic loss or a single allelic loss (HR = 1.9, $P = 0.002$; Figure 6). By contrast, patients with adenocarcinomas that had both p53 overexpression and allelic loss of 18q did not have a statistically significant worse survival as compared with no p53 overexpression and/or no allelic loss of 18q ($P = 0.12$).

Discussion

Adenocarcinomas in the distal esophagus and EGJ region share similar clinical and pathological features. The presence of Barrett esophagus is associated with in-

Figure 6. Kaplan-Meier survival curves of patients with adenocarcinoma in the distal esophagus and the EGJ region according to TNM stage (A) and allelic losses of chromosomes 17p and 18q (B). Survival was significantly better in patients with stage I disease than in patients with higher clinical stages. Allelic loss of 17p or 18q alone was marginally associated with survival (see Table 3). However, patients with allelic losses of both 17p and 18q in their cancers had significantly worse survival than patients with either one or no allelic loss ($P = 0.002$).

Table 2. Univariate Cox Proportional Hazards Ratios from Survival Analysis of Patients with Adenocarcinomas in the Distal Esophagus and Esophagogastric Junction

	Number	HR	95% CI	P value
Stage				
I	11	1.0		
II	24	7.6	1.8 to 33.1	0.007
III	42	12.2	2.9 to 51.6	<0.001
IV	15	20.1	4.4 to 92.7	<0.001
18q allelic loss				
No	25	1.0		
Yes	62	1.8	1.0 to 3.2	0.04
17p allelic loss				
No	22	1.0		
Yes	68	1.8	1.0 to 3.2	0.06
5q allelic loss				
No	45	1.0		
Yes	43	1.5	0.9 to 2.6	0.09
p53 overexpression				
No	41	1.0		
Yes	51	1.2	0.7 to 2.0	0.44
Differentiation				
Well/Moderate	71	1.0		
Poor	21	1.3	0.7 to 2.2	0.37
Barrett mucosa				
Absent	36	1.0		
Present	56	0.9	0.5 to 1.4	0.55
Preoperative Tx				
No	81	1.0		
Yes	11	1.0	0.5 to 1.9	0.91
Postoperative Tx				
No	68	1.0		
Yes	24	1.1	0.6 to 1.9	0.75

CI, confidence interval. Tx, treatment with chemotherapy and/or radiation.

creased risk of developing adenocarcinomas,^{6,36} and there is evidence that adenocarcinoma without Barrett mucosa may be due to tumor overgrowth of the precursor Barrett mucosa.^{26,37} The occurrence of similar alterations in the *p53* tumor suppressor gene in adenocarcinoma in the distal esophagus and EGJ suggests a common molecular pathogenesis.¹⁰ Additional support for the relationship between these types of cancers comes from the similar frequency of microsatellite instability and of allelic losses of chromosomes 17p, 18q, and 5q in adenocarcinomas with and without Barrett mucosa in our study.

In this study, we clearly demonstrated accumulation of genetic alterations in the morphological Barrett mucosa–columnar epithelial dysplasia–adenocarcinoma sequence.^{6,38} Allelic losses of 17p and 5q have been reported in premalignant Barrett lesions.^{12,39–41} In contrast to colorectal carcinoma, allelic loss of 17p (*p53*) appeared to precede allelic loss of 5q (*APC*) during neoplastic progression of esophageal adenocarcinoma.³⁹ The presence of allelic losses in nondysplastic Barrett mucosa may be due to allelic losses occurring before the development of recognizable morphological changes. 18q loss has been shown previously to occur frequently in esophageal adenocarcinomas.^{15,19,42} In our study, there was a higher percentage of allelic loss of both 18q and 17p as compared with 5q in Barrett mucosa and LGD. These findings suggest that 18q as well as 17p allelic losses may provide selective advantage to the

dysplastic columnar epithelial cell population in the Barrett mucosa–dysplasia–adenocarcinoma sequence. Therefore, alteration of tumor suppressor gene(s) on the long arm of chromosome 18 as well as the *p53* tumor suppressor gene on 17p may play an important early role in the development of esophageal adenocarcinoma.

As in colorectal tumorigenesis,⁴³ allelic losses of chromosomes in multiple foci of known tumor suppressor genes, including 17p (*p53* gene), 18q (*DCC*, *DPC4*, and *JV18-1*), 5q (*APC*), and 9p (*p16*), have been reported in esophageal adenocarcinomas.^{15–19} The high frequency of 18q allelic loss has not been associated with mutations in the *DPC4* tumor suppressor gene in esophageal adenocarcinoma,²² but the possible roles of *DCC* and *JV18-1* are not clear. The importance of *p53* is supported by the high frequency of overexpression of *p53* gene product in dysplastic Barrett mucosa^{7–8,10} and the strong association between 17p allelic loss and *p53* gene mutation in esophageal adenocarcinoma and Barrett mucosa in this and a previous study.⁴⁴ In addition, mutation of the *APC* gene on 5q has been identified in only 6% (1 of 18) of esophageal adenocarcinomas.²¹

Interestingly, allelic loss of 17p (2 cases), 18q (2 cases), or 5q (1 case) was occasionally present in dysplastic Barrett mucosa but not in associated adenocarcinoma, similar to *p53* overexpression in our previous study.¹⁰ These findings suggest that in some patients, adenocarcinoma develops from subclones in Barrett mucosa without these genetic alterations that appear to be important in the molecular pathogenesis of most esophageal adenocarcinomas. The discordance between dysplastic Barrett mucosa and adenocarcinoma cannot be explained by technical errors introduced by microdissection, because allelic loss of the other chromosomal arms was present in the adenocarcinoma in all five cases.

Table 3. Multivariate Regression Models Showing Hazards Ratios in Patients with Adenocarcinomas in the Distal Esophagus and Esophagogastric Junction

	Number	HR	95% CI	P value
Model 1				
Stage				
I	11	1.0		
II	21	9.0	2.0 to 40.4	0.004
III	40	14.2	3.3 to 61.1	<0.001
IV	15	20.0	4.2 to 94.7	<0.001
17p allelic loss				
No	22	1.0		
Yes	65	1.8	0.9 to 3.3	0.06
18q allelic loss				
No	25	1.0		
Yes	62	1.7	0.9 to 3.1	0.08
Model 2				
Stage				
I	11	1.0		
II	21	8.3	1.8 to 37.0	0.006
III	40	12.6	2.9 to 53.9	<0.001
IV	15	17.7	3.7 to 83.7	<0.001
17p and 18q allelic loss				
None or one	41	1.0		
Two	46	1.9	1.1 to 3.2	0.002

CI, confidence interval.

We found 5.4% (5 of 92) of adenocarcinomas in the distal esophagus and EGJ region were RER+, but RER was not found in Barrett mucosa either with or without dysplasia. The differing criteria used to define RER+ in this study (≥ 4 of 14 markers) and in the literature (more than one locus) can explain the lower rate of RER+ esophageal adenocarcinomas in this study.²⁴⁻²⁵ The lower frequency in Barrett mucosa than in adenocarcinoma in our study and the literature shows that when RER does develop, it occurs late in the dysplasia-adenocarcinoma sequence.²⁴ p53 overexpression was found in 3 of 5 RER+ esophageal adenocarcinomas, in marked contrast with RER+ colorectal carcinoma, which rarely has overexpression of p53 gene.⁴⁵ RER+ esophageal adenocarcinomas tended to have poor differentiation (80%, 4 of 5), as previously described in RER+ sporadic colorectal carcinomas (53%, 9 of 17).⁴⁵ RER+ cancers have been associated with a better prognosis in hereditary nonpolyposis colorectal carcinoma, sporadic colon carcinoma,⁴⁶ and gastric carcinoma.⁴⁷ We found a trend toward longer median survival for the 5 RER+ esophageal adenocarcinomas in this series. Future study of a larger number of patients is needed to clarify further the molecular features and survival in the subset of patients with RER+ esophageal adenocarcinoma.

The management of patients with high-grade columnar epithelial dysplasia in Barrett esophagus is controversial. The premalignant nature of HGD and the difficulty in detection of invasive carcinoma by endoscopy make prophylactic esophagectomy and ablative therapy considerations,⁴⁸⁻⁵¹ whereas some investigators recommend treatment when cancer is diagnosed.⁵² We found similar genetic alterations in HGD from prophylactic esophagectomy specimens and stage I adenocarcinomas. This finding further supports the premalignant nature of HGD and its utility as an indication for prophylactic therapy.

Survival after surgical resection in patients with symptomatic adenocarcinoma in the distal esophagus and EGJ region is poor. Early clinical stage is the most important prognostic factor in resected esophageal adenocarcinoma, as shown in this and previous studies,^{10,53} but molecular markers may also be helpful. Allelic loss of chromosome 17p ($P = 0.06$) and 18q ($P = 0.08$) each appeared to be marginally predictive of patient survival when adjusted for clinical stage, and there was no association of allelic loss of 17p or 18q with clinical stage or tumor differentiation. However, patients with adenocarcinoma characterized by allelic losses of both 17p and 18q had worse survival, with an HR of nearly 2 as compared with those with no or single allelic loss ($P = 0.002$). Therefore, evaluation of these allelic losses may be useful as an additional prognostic indicator. However, patients with adenocarcinomas that had both p53 overexpression and allelic loss of 18q did not have a statistically significantly worse survival. We evaluated the status of the p53 gene by both immunohistochemistry and 17p allelic loss, but not by DNA sequencing of all of the exons of the p53 gene due to the large number of cases studied. There is recognized discordance between p53 overexpression by immunohistochemistry and presence of p53 mutation,^{9,54} and allelic loss of 17p is more frequent than p53 overex-

pression and mutation.^{7,11} Overexpression of p53 tumor suppressor gene product was not a prognostic indicator in our previous study.¹⁰ Therefore, study of p53 gene mutation by DNA sequencing is needed to further clarify the prognostic implication of p53 gene mutations.

In conclusion, we have provided additional evidence that genetic alterations accumulate in the morphological dysplasia-adenocarcinoma sequence during esophageal carcinogenesis. RER was uncommon, but allelic loss of chromosome 18q as well as 17p occurred early, and these losses may play important roles in neoplastic progression. Furthermore, high-grade columnar epithelial dysplasia had a genetic profile similar to that of adenocarcinoma, supporting its importance as a premalignant lesion. Clinical stage was the most important prognostic indicator for patient survival after esophagectomy, but concomitant allelic losses of chromosomes 17p and 18q appear to predict a worse prognosis.

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