Mutations in Zinc-binding Domains of p53 as a Prognostic Marker of Esophageal-cancer Patients

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Some investigators have suggested that mutations of the *p53* gene may be molecular markers for poor prognosis of cancer patients, although others have reported conflicting results. We examined esophageal cancers from 138 patients to investigate whether mutational status of p53 could be correlated either with prognosis or with response to chemotherapy or radiation. We detected p53 mutations in the tumors of 78 (56.5%) patients. Kaplan-Meier analysis showed that these 78 patients tended to have shorter survival times and greater resistance to either form of therapy than patients whose tumors carried two wild-type p53 alleles. The difference became more evident when we focused on mutations in zinc-binding domains of p53 (L2 and L3); the prognosis was significantly poorer among the 29 patients with tumors in this category than among patients whose tumors had no p53 mutations, or p53 mutations outside L2 or L3 (*P*=0.0060). Moreover, those tumors as a group were more resistant to chemotherapy or radiation than the others (*P*=0.0105). Our results underscore the importance of the zinc-binding domains of p53 with respect to clinical prognosis for patients with esophageal carcinomas.

Key words: p53 — Mutation — Zinc-binding domains — Esophageal cancer — Prognosis

Recent developments in cancer research have confirmed that carcinomas arise when genetic and epigenetic alterations of multiple genes accumulate in human cells. Some of those genes are likely to play crucial roles in the development of resistance to chemotherapeutic agents and radiation. The tumor-suppressor gene *p53*, whose normal role is to induce cell-cycle arrest or trigger apoptosis in response to DNA damage, is often mutated in a variety of cancer types. A significant correlation between p53 mutation and response to chemotherapy and radiation therapy has been demonstrated by studies *in vivo* and *in vitro*.[1, 2] Furthermore, mutations in certain parts of the *p53* gene lead to critical structural changes in the protein product, and those changes are associated with shorter survival of patients with breast or colorectal cancers.[3, 4] With regard to esophageal cancers, mutations of the *p53* gene have been reported in 38–69% of the tumors examined to date.[5–7] However, the claim that p53 mutation can be a general prognostic indicator, or a predictor for response to therapy, remains controversial.[8, 9]

In the study reported here we examined the mutational status of p53 in esophageal cancers from 138 patients, and investigated the correlation of mutations with either the patients' prognoses or their response to chemotherapy or radiation. We determined that mutations in a specific region of the *p53* gene may be predictive of both clinical outcome and response to these types of therapy for patients with esophageal cancers.

MATERIALS AND METHODS

Patients and tumor samples Clinicopathological characteristics were documented for 138 patients who underwent surgery for esophageal cancers at the Kurume University School of Medicine between 1989 and 1996 (Table I). Curative operation was performed with locoregional lymphadectomy. The mean age at surgery was 62.2 years (range, 42–85 years). The median period of follow-up was 28 months (range, 1–104 months), established as the time between surgery and either death or the last update (May 18, 1998). Of the 138 patients, 47 survived to the close of the study with or without recurrent disease, and 91 died. For calculation of survival time, only cancer-related deaths were considered; data on the 13 patients who died from other causes were excluded. Histologically, 136 cases of 138 were squamous cell carcinoma, and the others were undifferentiated carcinoma and basaloid carcinoma. Genomic DNA was extracted from resected esophageal tumors and from corresponding normal tissues. All of the

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Table I. Correlation between p53 Mutation and Clinicopathological Features

	Total	p53 mutation (%)	χ^2 test
	138	78	
Sex			
male	129	74 (57.3)	P=0.44
female	9	4 (44.4)	
Histological grade			
well[a]	47	30 (63.8)	P=0.44
moderate[b]	66	34 (51.5)	
poor[c]	23	13 (56.5)	
others[d]	2	1 (50.0)	
Depth of invasion			
mucosa	3	2 (66.7)	P=0.21
sub-mucosa	14	5 (35.7)	
muscularis propria	13	7 (53.8)	
extra-mural	108	64 (59.2)	
pTNM stage			
0	3	2 (66.7)	P=0.76
I	10	3 (30.0)	
IIA	5	3 (60.0)	
IIB	17	9 (52.9)	
III	70	38 (54.3)	
IV	33	23 (69.7)	
Vascular invasion			
positive	120	70 (58.3)	P=0.07
negative	18	8 (44.4)	
Double cancer			
+	32	19 (59.3)	P=0.71
−	106	59 (55.6)	

	p53 mutation	Without mutation	Unpaired t test
Age (years)	62.2±8.3	61.1±7.6	P=0.42
Number of LNs metastasis	4.3±0.6	4.0±0.6	P=0.72
Tumor size (cm)	7.5±3.3	8.6±6.5	P=0.19

a) Well differentiated squamous cell carcinoma.
b) Moderately differentiated squamous cell carcinoma.
c) Poorly differentiated squamous cell carcinoma.
d) Undifferentiated carcinoma and basaloid carcinoma.

Table II. Primer Sequences for Exons 5–8 of p53

Primer	Nucleotide sequence
5F	5′-CTT GTG CCC TGA CTT TCA AC -3′
5R	5′-AGC CCT GTC GTC TCT CCA G -3′
6F	5′-TGA TTC CTC ACT GAT TGC TCT -3′
6R	5′-CCA GAG ACC CCA GTT GCA AAC -3′
7F	5′-TCT TGG GCC TGT GTT ATC TC -3′
7R	5′-GCA CAG CAG GCC AGT GTG C -3′
8F	5′-GCT TCT CTT TTC CTA TCC TGA -3′
8R	5′-ACC GCT TCT GTT CCT GCT TG -3′

specimens had been snap-frozen in liquid nitrogen and stored at −80°C using a protocol described previously.[10]

PCR, SSCP, and DNA sequencing Because most studies of *p53* gene mutations are limited to exons 5–8, only mutations within this region of gene were analyzed. We used four sets of primers to screen exons 5–8 (Table II). A series of SSCP analyses, followed by direct sequencing, was carried out with only slight modification to the method reported by Soong and Iacopetta.[11] Gels were stained with fluorescent dye (SyBR Green II, TaKaRa, Japan) and scanned by a Fluorimager (Molecular Dynamics, Sunnyvale, CA). PCR products that showed aberrant bands on SSCP gels were purified and sequenced with an ABI 377 Autosequencer (Perkin-Elmer, Foster City, CA). PCR-direct sequencing experiments were repeated independently to confirm mutations. When no mutations could be detected by the primary sequencing, DNA was extracted from aberrant bands that had been excised from the SSCP gels. These DNAs served as templates for PCR reactions using the same primers as in the first amplification experiments. The products were purified and sequenced in the same way.

Statistical analysis The χ^2 test and the unpaired t test were used to calculate statistical associations between various clinicopathological features and the presence or absence of p53 alterations. Kaplan-Meier curves were constructed to reveal clinical outcomes and responses to chemotherapy and/or radiation between categories of patients.[12] Comparisons of survival curves were performed using the log-rank test. An association was considered statistically significant when the *P* value was below 0.05.

RESULTS

p53 mutations in esophageal cancers Mutations of the *p53* gene were detected in tumors from 78 (56.5%) of the 138 patients examined (Table III). Among them, 66 (84.6%) were point mutations, of which 59 were missense and the other seven nonsense mutations. Forty-seven (71.2%) of the 66 point mutations were transitions; G-to-A changes were predominant (35 cases) and 22 of them had occurred at a CpG site. Of the remaining 12 tumors, two (2.6%) contained p53 sequences with insertions (6 bp or 9 bp, respectively), and ten (8.9%) showed deletions of 2 bp (4 cases), 1 bp (3 cases), 3 bp (2 cases) or 7 bp (1 case).

The 78 mutations were distributed over 43 distinct codons. We found 27 (34.1%) mutations in exon 5, 18 (22.7%) in exon 6, 17 (22.7%) in exon 7, and 17 (22.7%) in exon 8. Among the 78 mutations, 46 (58.9%) had occurred within conserved regions II–V, and 33 of those (42.3%) were within zinc-binding domains (regions L2 and L3; Fig. 1). These domains correspond to codons

Table III. Characteristics of p53 Mutation in Esophageal Cancer Patients

Patient ID	Sex	Age	Exon	Codon	Base change	AA change	Conserved region	Zinc-binding domain	Outcome	Survival period (months)
89-03	M	67	5	c131	AAA-TAC	Lys-Tyr	II		death	17
89-04	M	85	7	c251	ATC-AGC	Ile-Ser	IV	L3	death	5
89-06	M	60	7	c244		9 bp insertion	IV	L3	death	22
89-14	M	62	8	c278	CCT-CTT	Pro-Leu	V		death	3
89-18	M	59	8	c282	CGG-TGG	Arg-Trp	V		death	6
89-21	M	63	6	c214	CAT-CGT	His-Arg			death	9
89-22	M	54	8	c271		6 bp insertion	V		survival	103
89-25	M	51	8	c272	GTG-ATG	Val-Met	V		death	8
89-27	F	76	7	c245	GGC-GCC	Gly-Ala	IV	L3	death	17
89-28	F	66	5	c153	CCC-CC	1 bp deletion			death	19
89-31	M	56	5	c175	CGC-CAC	Arg-His	III	L2	death	20
89-33	M	67	5	c173	GTG-ATG	Val-Met	III	L2	death	35
89-36	M	44	5	c173	GTG-ATG	Val-Met	III	L2	death	6
89-46	M	64	5	c146	TGG-TGA	Trp-stop			survival	97
90-13	M	72	8	c280	AGA-	3 bp deletion	V		death	18
90-16	M	60	7	c248	CGG-CAG	Arg-Gln	IV	L3	death	6
90-19	M	58	5	c138	GCC-GTA	Ala-Val	II		death	28
90-22	M	61	6	c220	TAT-TGT	Tyr-Cys			death	44
90-23	M	64	8	c273	CGT-TGT	Arg-Cys	V		death	4
90-24	M	74	5	c175	CGC-CAC	Arg-His	III	L2	death	10
90-25	M	63	7	c248	CGG-CAG	Arg-Gln	IV	L3	death	5
90-28	F	72	6	c212	TTT-T	2 bp deletion			death	22
90-30	M	57	5	c163	TAC-TAA	Tyr-stop		L2	death	12
90-31	M	56	6	c299	TGT-TAT	Cys-Tyr			death	12
90-36	M	59	5	c175	CGC-CAC	Arg-His	III	L2	death	13
91-05	M	66	5	c154	GGC-GTC	Gly-Val			death	17
91-06	M	61	6	c192	CAG-TAG	Gln-stop		L2	death	27
91-09	M	69	5	c173	GTG-TTG	Val-Leu	III	L2	death	18
91-17	M	58	5	c183	TCA-TGA	Ser-stop		L2	survival	82
91-21	M	62	8	c273	CGT-CAT	Arg-His	V		survival	79
91-25	M	60	6	c194	CTT-CGT	Leu-Arg		L2	death	16
91-29	M	70	6	c214	CAT-CGT	His-Arg			death	55
91-30	M	65	6	c195	ATC-TTC	Ile-Phe		L2	death	11
91-33	M	45	6	c220	TAT-TGT	Tyr-Cys			death	23
91-35	M	58	5	c157	GTC-TC	1 bp deletion			death	19
92-03	M	45	5	c154	GGC-GTC	Gly-Val			death	7
92-08	M	55	7	c257	CTG-CCG	Leu-Pro	IV		death	5
92-12	M	56	5	c176	TGC-TCC	Cys-Ser	III	L2	death	24
92-13	M	70	8	c298	GAG-TAG	Glu-stop			death	11
92-15	M	70	6	c209	AGA-A	2 bp deletion			death	5
92-18	M	61	7	c237	ATG-GTG	Met-Val	IV	L3	death	5
92-23	M	67	5	c179	CAT-AAT	His-Asn	III	L2	death	3
92-30	M	64	7	c248	CGG-CAG	Arg-Gln	IV	L3	death	6
92-36	M	68	8	c278	CCT-TCT	Pro-Ser	V		survival	63
92-42	M	70	7	c250	CCC-CTC	Pro-Leu	IV	L3	survival	62
92-43	M	70	6	c205	TAT-TGT	Tyr-Cys			death	9
94-06	M	64	7	c245	GGC-GTC	Gly-Val	IV	L3	death	6
94-10	M	55	5	c179	CAT-CGT	His-Arg	III	L2	death	4
94-11	M	60	5	c176	TGC-TAC	Cys-Tyr	III	L2	death	6
94-12	M	43	5	c176	TGC-TTC	Cys-Phe	III	L2	death	24
94-15	M	66	8	c278	CCT-TCT	Pro-Ser	V		survival	45
94-22	M	65	7	c248	CGG-CAG	Arg-Gln	IV	L3	death	18
94-23	M	61	6	c205	TAT-TGT	Tyr-Cys			survival	43

Table III. Continued

| Patient ID	Sex	Age	Exon	Codon	Base change	AA change	Conserved region	Zinc-binding domain	Outcome	Survival period (months)
94-24	F	72	6	c215	AGT-AGG	Ser-Arg			survival	42
94-26	M	68	5	c156	CGC-	3 bp deletion			death	36
94-27	M	56	6	c209	AGA-A	2 bp deletion			death	4
95-06	M	64	8	c280	AGA-AGT	Arg-Ser	V		death	8
95-08	M	63	6	c220	TAT-TGT	Tyr-Cys			survival	32
95-13	M	77	7	c229		7 bp deletion			death	3
95-15	M	75	6	c190	CCT-CTT	Pro-Leu		L2	death	1
95-19	M	48	5	c175	CGC-CAC	Arg-His	III	L2	survival	30
95-23	M	59	5	c175	CGC-CAC	Arg-His	III	L2	death	2
95-27	M	66	6	c205	TAT-TGT	Tyr-Cys			death	16
95-28	M	63	5	c153	GGC-GC	1 bp deletion			survival	27
95-30	M	71	8	c278	CCT-TCT	Pro-Ser	V		survival	26
95-33	M	50	6	c205	TAT-TGT	Tyr-Cys			survival	25
95-34	M	67	8	c278	CCT-TCT	Pro-Ser	V		survival	25
96-01	M	69	5	c146	TGG-TAG	Trp-stop			survival	24
96-04	M	56	5	c144	CAG-TAG	Gln-stop			death	15
96-08	M	67	7	c238	TGT-TAT	Cys-Tyr	IV	L3	survival	21
96-09	M	69	8	c280	AGA-AAA	Arg-Lys	V		survival	21
96-23	M	47	8	c280	AGA-AGT	Arg-Ser	V		death	5
96-24	M	60	7	c245	GGC-GAC	Gly-Asp	IV	L3	survival	18
96-26	M	57	7	c248	CGG-CAG	Arg-Gln	IV	L3	survival	17
96-36	M	55	7	c248	CGG-TGG	Arg-Trp	IV	L3	survival	15
96-37	M	61	7	c255	ATC-TTC	Ile-Phe	IV		survival	14
96-38	M	46	8	c300	CCC-G	2 bp deletion			survival	13
96-39	M	75	8	c282	CGG-TGG	Arg-Trp	V		survival	13
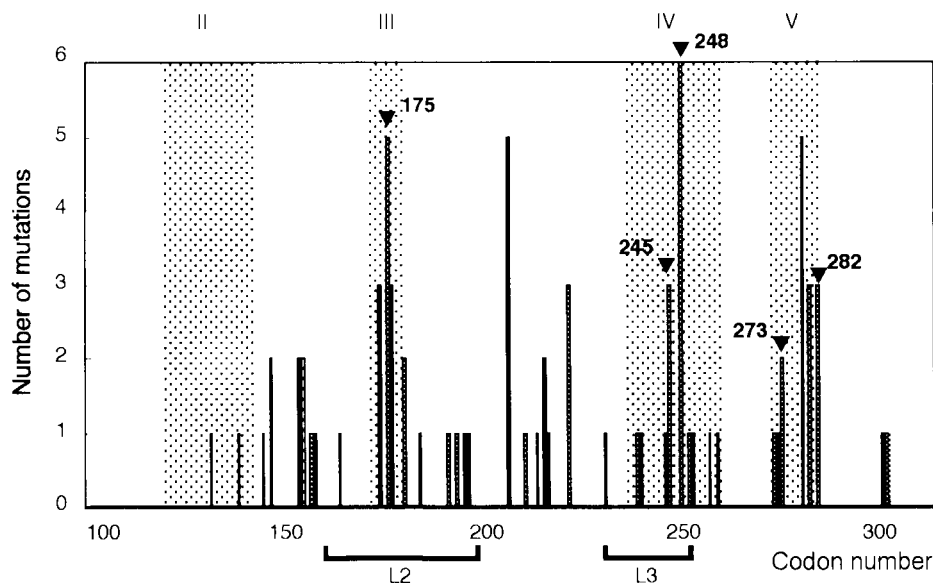
Fig. 1. Frequency and locations of p53 mutations. L2, L3, zinc-binding domains; II, III, IV, V, conserved region. Downward arrowheads indicate hot spots.
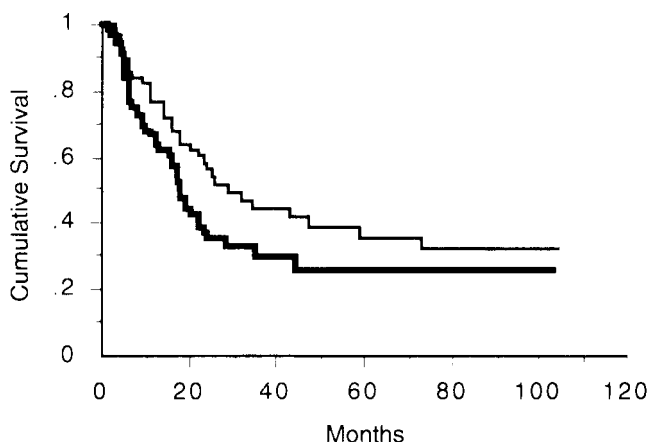
Fig. 2. Adjusted survival curves for patients with p53 mutations in their tumors (heavy line, $n=69$) and patients without p53 mutations (thin line, $n=56$). The difference was not statistically significant ($P=0.0807$).
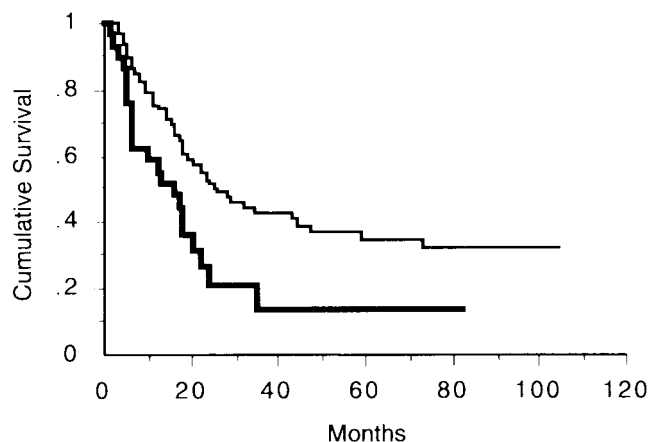

Fig. 3. Adjusted survival curves for 29 patients whose tumors carried p53 mutation within zinc-finger domains L2 or L3 (heavy line) and for 96 other patients (thin line) (p53 mutations outside zinc-finger domains or with no mutations in this gene). The difference was statistically significant ($P=0.0060$).

Table IV. Patients Who Received Chemotherapy and/or Radiation

Patient ID	Radiation (Gy)		Chemotherapy		p53	Codon	Outcome	Survival period (months)
89-02	60	post[a]	CDDP+5FU 2 week	post	WT		death	9
89-03	60	post	CDDP+5FU 2 week	post	MT	c131	death	17
89-12	—	—	CDDP+VDS 2 week	post	WT		survival	104
89-14	—	—	CDDP+5FU 2 week	post	MT	c278	death	3
89-15	50	post	CDDP+5FU+254S	post	WT		death	11
89-21	—	—	CDDP+5FU 2 week	post	MT	c214	death	9
89-22	—	—	CDDP+5FU 2 week	post	MT	c271	survival	103
89-24	—	—	CDDP+5FU 2 week	post	WT		death	14
89-25	30	post	CDDP+5FU 2 week	post	MT	c272	death	8
89-27	—	—	CDDP+5FU 2 week	post	MT	c245	death	17
89-31	—	—	254S+5FU 2 week	post	MT	c175	death	20
89-32	50	post	CDDP+5FU 2 week	post	MT	c173	survival	101
89-33		—	CDDP+5FU 2 week	post	WT		death	35
89-36	—	—	CDDP+5FU 3 week	post	MT	c173	death	6
89-41	—	—	CDDP+5FU 3 week	post	WT		death	29
89-43	—	—	CDDP+VDS 2 week	post	WT		death	14
90-05	—	—	CDDP+VDS	post	WT		death	59
90-09	—	—	CDDP+VDS	post	WT		survival	95
90-12	50	post	CDDP+5FU	post	WT		death	3
90-13	50	post	254S+5FU	post	MT	c280	death	18
90-16	50	post	CDDP+5FU	post	MT	c248	death	6
90-19	—	—	CDDP+VP16	post	MT	c138	death	28
90-21	—	—	CDDP+VDS	post	MT	c220	survival	91
90-22	50	post	CDDP+5FU 2 week	post	WT		death	44
90-23	—	—	254S+5FU	post	MT	c273	death	4
90-24	50	post	—	—	MT	c175	death	10
90-25	20	post	CDDP+5FU	post	MT	c248	death	5
90-27	—	—	CDDP+VDS 2 week	post	MT	c163	survival	89
90-28	—	—	CDDP+VDS 2 week	post	MT	c212	death	22

Table IV.   Continued

Patient ID	Radiation (Gy)		Chemotherapy		p53	Codon	Outcome	Survival period (months)
90-30	50	post	CDDP+5FU	post	WT		death	12
90-31	—	—	CDDP+5FU	post	MT	c299	death	12
90-34	50	post	CDDP+5FU	post	WT		death	23
91-05	—	—	CDDP+VDS 2 week	post	MT	c154	death	17
91-07	—	—	CDDP+5FU 2 week	post	WT		death	24
91-09	50	post	CDDP 2 week	post	MT	c173	death	18
91-13	—	—	CDDP+5FU 2 week	post	WT		survival	83
91-21	—	—	CDDP+5FU	post	MT	c273	survival	79
91-25	—	—	CDDP+5FU 2 week	post	MT	c194	death	16
91-26	—	—	CDDP+5FU 2 week	post	WT		death	4
91-35	—	—	CDDP+5FU 2 week	post	MT	c157	death	19
91-41	—	—	CDDP+5FU 2 week	post	WT		death	16
92-03	—	—	CDDP+5FU 2 week	post	MT	c154	death	7
92-08	40	post	—	—	MT	c257	death	5
92-16	—	—	CDDP+5FU 3 week	post	WT		death	43
92-27	50	post	CDDP+5FU 2 week	post	WT		death	25
92-30	—	—	CDDP+5FU	post	MT	c248	death	6
92-33	50	post	CDDP+5FU	post	WT		death	5
92-36	—	—	CDDP+5FU 2 week	post	MT	c278	survival	63
92-40	50	post	CDDP+5FU	post	WT		death	16
92-43	50	post	CDDP+5FU	post	MT	c205	death	9
93-06	50	post	CDDP+5FU	post	WT		death	18
93-15	—	—	CDDP+5FU 2 week	post	WT		death	47
93-29	—	—	CDDP+5FU	post	WT		survival	55
93-36	—	—	CDDP+5FU 2 week	post	WT		death	34
94-12	—	—	CDDP+5FU 2 week	post	MT	c176	death	24
94-22	—	—	CDDP+5FU 2 week	post	MT	c248	death	18
94-23	—	—	CDDP+5FU 2 week	post	MT	c205	survival	43
95-06	60	post	CDDP+5FU 2 week	post	MT	c280	death	8
95-07	50	post	—	—	WT		death	11
95-09	—	—	CDDP+5FU 2 week	post	WT		survival	32
95-15	52.3	pre[b]	—	—	MT	c190	death	1
95-23	60	pre	CDDP+5FU 2 week	pre	MT	c175	death	2
95-27	50	post	CDDP+5FU	post	MT	c205	death	16
95-28	50	post	CDDP+5FU	post	MT	c153	survival	27
95-30	50	post	CDDP+5FU	post	MT	c278	survival	26
95-33	50	intra[c]	CDDP+5FU	post	MT	c205	survival	25
95-34	50	intra	CDDP+5FU	post	MT	c278	survival	25
95-37	—	—	CDDP+5FU 2 week	pre	WT		survival	24
96-04	—	—	CDDP+5FU	post	MT	c144	death	15
96-08	—	—	CDDP+5FU	post	MT	c238	survival	21
96-23	50	post	—	—	MT	c280	death	5
96-27	40	pre	CDDP+5FU	post	WT		death	3
96-34	60	post	CDDP+FT	post	WT		survival	16
96-36	—	—	CDDP+5FU	post	MT	c248	survival	15

a) Postoperative.
b) Preoperative.
c) Intraoperative.
CDDP, cisplatin; 5FU, 5-fluorouracil; VDS, vindesine; VP16, etoposide; 254S, (glycolato-O,O′)-diammineplatinum(II);
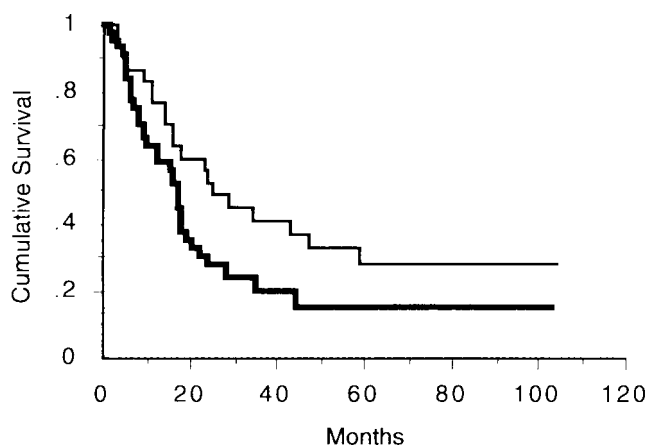FT, Tegafur.

Fig. 4. Adjusted survival curves for 74 chemotherapy- and/or radiation-treated patients with (heavy line, $n=44$) or without (thin line, $n=30$) p53 mutations in their esophageal tumors. The difference was not statistically significant ($P=0.0645$).
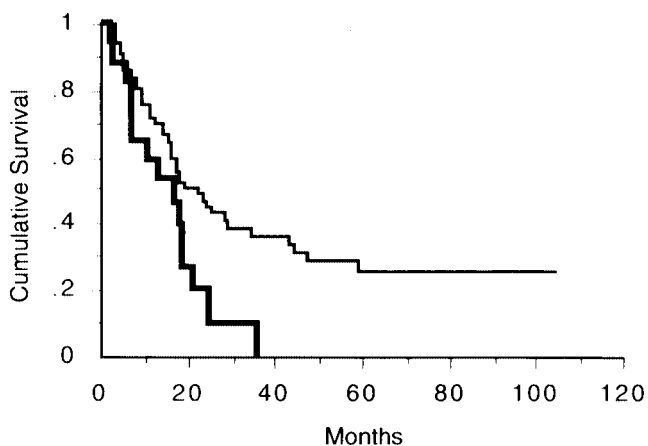

Fig. 5. Adjusted survival curves for 74 chemotherapy- and/or radiation-treated patients: those whose tumors carried *p53* gene mutations within zinc-binding domains (L2 and L3, heavy line, $n=17$) vs. all other treated patients (those with p53 mutations outside zinc-finger domains plus those without mutations, thin line, $n=57$). The difference between these groups was statistically significant ($P=0.0105$).

163–195 and 236–251, according to published analyses of the crystal structure of the p53-DNA complex.[13]

p53 mutations and clinicopathological features Table I identifies the clinicopathological characteristics of the 138 patients analyzed and the frequency of p53 mutation in each subdivided group. We found no significant correlation between the mutational status of p53 and any of the clinicopathological parameters.

After exclusion of 13 patients who died from causes unrelated to esophageal cancer, we divided the remaining 125 patients into two groups, i.e. 56 patients whose tumors contained no detectable p53 mutations, and 69 whose tumors did reveal p53 mutations. Fig. 2 shows the Kaplan-Meier curves for the adjusted survival of each group; the curves indicate a tendency toward shorter survival of patients with p53 mutations in their tumors, but not to the level of statistical significance ($P=0.0807$).

We then divided the 69 tumors with p53 mutations according to the intragenic locations where the alterations had occurred. The group (29 patients) in which mutations fell within zinc-binding domains L2 and L3 showed a shorter survival time than the group of 40 patients whose tumors had p53 mutations outside those two domains ($P=0.0492$, data not shown). Furthermore, comparison of the patients whose tumors contained the p53 mutation within the L2 and L3 domains with all other patients (those with mutations outside L2 and L3 and those with no p53 mutations in their tumors) revealed a significant difference in survival ($P=0.0060$; Fig. 3). The two groups did not differ significantly with respect to clinicopathological features.

Mutations of p53 and response to chemotherapy and/or radiation Among the 125 patients in the panel, 74 had received either chemotherapy or radiation, or both, before or after surgery (Table IV). We investigated whether the presence or absence of a p53 mutation in the tumor might influence the response of esophageal-cancer patients to chemotherapy or radiation therapy by dividing these patients again into two groups; 30 patients without and 44 patients with p53 mutations. Although a tendency toward shorter survival in the p53-mutated group was suggested by the Kaplan-Meier curves (Fig. 4), it was not statistically significant ($P=0.0645$). Then we compared the 17 treated patients whose mutations lay within the L2 or L3 zinc-binding domains with the remaining 57 patients (30 having no p53 mutations and 27 with mutations outside of these domains; Fig. 5). Although the two groups did not differ significantly in clinicopathological features, the patients whose tumors contained mutations within one of the zinc-binding domains exhibited shorter survival; all died within 35 months after surgery ($P=0.0105$).

DISCUSSION

In this study we detected p53 mutations in 78 of 138 esophageal cancers examined. This frequency (56.4%) is as high as the highest frequency reported by Casson *et al.*,[6] who screened exons 4–10 of the p53 gene by means of single strand conformation polymorphism (SSCP) analysis and DNA sequencing. Greenblatt *et al.*[14] reported that 87% of all mutations are within exons 5–8. Hence it is difficult to evaluate the association between p53 mutation

outside of exons 5–8 and clinicopathological phenotype. Previous studies involving colorectal cancers, breast cancers, and non-small cell lung cancers had suggested a correlation between p53 mutations and poor prognosis among cancer patients,[15–17] but reports from other groups had found no such correlation.[18, 19] With regard to esophageal cancers, the relationship of p53 status to prognosis also remains controversial.[8, 9] Among the relatively large number of patients we examined, it appeared that those whose tumors carried p53 mutations tended to have poorer prognoses than the others. However, when we divided the patients with p53 mutations according to the intragenic locations of the alterations, the difference became more evident; tumors that contained p53 mutations within the L2 or L3 zinc-binding domains conferred statistically worse prognoses than the other tumors. Therefore we concluded that mutations within the zinc-binding domains of p53 should be useful markers for predicting clinical outcomes for patients with esophageal cancer.

Breast tumors carrying p53 mutations within zinc-binding domains also confer a decrease in survival time relative to tumors with mutations in other domains.[4] Since the L2 and L3 domains are critical for binding to specific DNA sequences in p53-target genes, mutations affecting these domains can change the structural conformation of the protein in such a way as to abrogate its ability to bind target molecules. Using p53-mutant cell lines, Rolley et al.[20] confirmed that not all mutations of p53 render the protein completely dysfunctional in this respect. Furthermore, several reports showed that different mutations within the DNA-binding domain caused different patterns of transactivation of the p53-target genes including p21, Bax, GADD45 and IGF-BP3 in vitro.[21] These data suggest that different mutations in vivo also may result in different biological properties of cancer tissues.

The results of many in vitro and in vivo studies have suggested that p53 may play a critical role in cell death in response to cytotoxic agents, UV light, and γ-irradiation.[1, 2] Furthermore, cells with mutated p53 genes often become resistant to such therapies.[22, 23] Several clinical studies involving breast and colorectal cancers have also indicated that the mutational status of p53 can help to predict response to chemotherapy and radiation.[24, 25] When we compared esophageal cancers with and without p53 mutations, we found no statistically significant difference in the survival of patients given chemotherapy and/or radiation therapy unless the mutations had occurred within zinc-binding domains; treated patients whose tumors contained p53 mutations within one of these domains all died within 35 months ($P=0.0105$). The results implied that esophageal tumors having p53 mutations within zinc-binding domains are likely to be more resistant to chemotherapy and/or radiation therapy than other tumors arising in the same type of tissue.

Most investigations of the relationship between p53 status and prognosis of cancers have compared patients in whose tumors the p53 mutation was either present or absent, without reference to the intragenic locations of the mutations. The controversies arising from the disparate results of various studies may reflect differences in methods or skill in mutational analysis, and/or differences in the distribution of the p53 mutations. Our data partially support previous reports of a positive association, and suggest as well that detection of mutations within zinc-binding domains of p53 could be useful for predicting prognosis and sensitivity to therapy among patients with esophageal cancer. However, since several different therapeutic protocols had been followed in our panel of patients, we cannot draw definitive conclusions for such a correlation until further studies can be undertaken with standardized treatment regimens.

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