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
p53 Mutations in Esophageal Cancers

Table I. Correlation between p53 Mutation and Clinicopathological Features

|                        | Total | p53 mutation (%) | \( \chi^2 \) test |
|------------------------|-------|------------------|-------------------|
| 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)        |                   |
| pTNN stage             |       |                  |                   |
| 0                      | 3     | 2 (66.7)         | \( P = 0.76 \)    |
| I                      | 10    | 3 (30.0)         |                   |
| IIA                    | 5     | 3 (60.0)         |                   |
| IIIB                   | 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)        |                   |

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

| Primer | Nucleotide sequence     |
|--------|-------------------------|
| 5F     | 5'-CTT GTG CCC TGA CTT TCA -3' |
| 5R     | 5'-AGC CCT 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 CTC CTT CTA TCC TGA -3' |
| 8R     | 5'-ACC GCT TCT TGT CTC CCT GCT TG -3' |

Table I. Correlation between p53 Mutation and Clinicopathological Features

- **Sex**: male 129 (74%), female 9 (44%).
- **Histological grade**: well\(^a\) 47 (30%), moderate\(^b\) 66 (34%), poor\(^c\) 23 (13%), others\(^d\) 2 (1%).
- **Depth of invasion**: mucosa 3 (2%), sub-mucosa 14 (5%), muscularis propria 13 (7%), extra-mural 108 (64%).
- **pTNN stage**: 0 3 (2%), I 10 (3%), IIA 5 (3%), IIIB 17 (9%), III 70 (38%), IV 33 (23%).
- **Vascular invasion**: positive 120 (70%), negative 18 (8%).
- **Double cancer**: + 32 (19%), – 106 (59%).

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

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

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  | 7    | c271  | 6 bp insertion | V       |                 |                     | survival | 103                   |
| 89-24      | 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 | V             |                     | 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  | GCC-GTC     | Gly-Val   |                 |                     | death   | 17                     |
| 91-06      | M   | 61  | 6    | c192  | CAG-TAG     | Gin-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  | GCC-GTC     | Gly-Val   |                 |                     | death   | 7                      |
| 92-08      | M   | 55  | 7    | c257  | GCT-CGG     | 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-ATG     | 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-CCT     | 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  | GCC-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-CCT     | 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-        |           |                  |                     |         | 36                       |
| 94-27      | M   | 56  | 6    | c209  | AGA-A       |           |                  |                     |         | 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  |             |           |                  |                     |         | 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\(^{+}\) | CDDP+5FU 2 week    | post| WT    | death   | 9                        |
| 89-03      | 60 post        | CDDP+5FU 2 week    | post| MT    | c131    | 17                       |
| 89-12      | — —            | CDDP+VDS 2 week    | post| WT    | survival| 104                     |
| 89-14      | — —            | CDDP+5FU 2 week    | post| MT    | c278    | 3                       |
| 89-15      | 50 post        | CDDP+5FU+254S      | post| WT    | death   | 11                      |
| 89-21      | — —            | CDDP+5FU 2 week    | post| MT    | c214    | 9                       |
| 89-22      | — —            | CDDP+5FU 2 week    | post| MT    | c271    | 103                     |
| 89-24      | — —            | CDDP+5FU 2 week    | post| WT    | death   | 14                      |
| 89-25      | 30 post        | CDDP+5FU 2 week    | post| MT    | c272    | 8                       |
| 89-27      | — —            | CDDP+5FU 2 week    | post| MT    | c245    | 17                      |
| 89-31      | — —            | 254S+5FU 2 week    | post| MT    | c175    | 20                      |
| 89-32      | 50 post        | CDDP+5FU 2 week    | post| MT    | c173    | 101                     |
| 89-33      | — —            | CDDP+5FU 2 week    | post| WT    | death   | 35                      |
| 89-36      | — —            | CDDP+5FU 3 week    | post| MT    | c173    | 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    | 18                      |
| 90-16      | 50 post        | CDDP+5FU           | post| MT    | c248    | 6                       |
| 90-19      | — —            | CDDP+VP16          | post| MT    | c138    | 28                      |
| 90-21      | — —            | CDDP+VDS           | post| MT    | c220    | 91                      |
| 90-22      | 50 post        | CDDP+5FU 2 week    | post| WT    | death   | 44                      |
| 90-23      | — —            | 254S+5FU           | post| MT    | c273    | 4                       |
| 90-24      | 50 post        | — —                | post| MT    | c175    | 10                      |
| 90-25      | 20 post        | CDDP+5FU           | post| MT    | c248    | 5                       |
| 90-27      | — —            | CDDP+VDS 2 week    | post| MT    | c163    | 89                      |
| 90-28      | — —            | CDDP+VDS 2 week    | post| MT    | c212    | 22                      |
Table IV. Continued

| Patient ID | Radiation (Gy) | Chemotherapy | p53 | Codon | Outcome | Survival period (months) |
|------------|----------------|--------------|-----|-------|---------|-------------------------|
| 90-30      | 50 post        | CDDP+5FU     | post WT | c299 | death 12 |                          |
| 90-31      | —              | CDDP+5FU     | post MT | c299 | death 12 |                          |
| 90-34      | 50 post        | CDDP+5FU     | post WT | c299 | death 23 |                          |
| 91-05      | —              | CDDP+VDS 2 week | post MT | c154 | death 17 |                          |
| 91-07      | —              | CDDP+5FU 2 week | post WT | c173 | death 18 |                          |
| 91-09      | 50 post        | CDDP 2 week  | post MT | c278 | survival 83 |                      |
| 91-13      | —              | CDDP+5FU 2 week | post WT | c278 | death 16 |                          |
| 91-16      | —              | CDDP+5FU 3 week | post WT | c278 | death 25 |                          |
| 92-30      | 50 post        | CDDP+5FU     | post WT | c278 | death 5  |                          |
| 92-33      | 50 post        | CDDP+5FU     | post WT | c278 | death 6  |                          |
| 92-36      | —              | CDDP+5FU 2 week | post MT | c278 | survival 63 |                       |
| 92-40      | 50 post        | CDDP+5FU     | post WT | c278 | death 16 |                          |
| 92-43      | 50 post        | CDDP+5FU     | post WT | c278 | death 9  |                          |
| 93-06      | 50 post        | CDDP+5FU     | post MT | c278 | survival 47 |                        |
| 93-15      | —              | CDDP+5FU 2 week | post WT | c278 | death 11 |                          |
| 93-29      | —              | CDDP+5FU     | post WT | c278 | survival 55 |                        |
| 94-12      | —              | CDDP+5FU 2 week | post MT | c176 | death 34 |                          |
| 94-22      | —              | CDDP+5FU 2 week | post MT | c248 | death 24 |                          |
| 94-23      | —              | CDDP+5FU 2 week | post MT | c248 | death 18 |                          |
| 95-06      | 60 post        | CDDP+5FU 2 week | post MT | c205 | survival 43 |                      |
| 95-07      | 50 post        | —             | — MT | c205 | death 8  |                          |
| 95-09      | —              | CDDP+5FU 2 week | post WT | c205 | survival 32 |                      |
| 95-15      | 52.3 pre       | —             | — MT | c175 | death 1  |                          |
| 95-23      | 60 pre         | CDDP+5FU 2 week | post MT | c175 | death 2  |                          |
| 95-27      | 50 post        | CDDP+5FU     | post MT | c205 | death 16 |                          |
| 95-28      | 50 post        | CDDP+5FU     | post MT | c205 | death 16 |                          |
| 95-30      | 50 post        | CDDP+5FU     | post MT | c205 | death 26 |                          |
| 95-33      | 50 intra       | CDDP+5FU     | post MT | c205 | survival 25 |                     |
| 95-34      | 50 intra       | CDDP+5FU     | post MT | c205 | survival 25 |                     |
| 95-37      | —              | CDDP+5FU 2 week | post MT | c205 | 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 | c280 | death 3  |                          |
| 96-34      | 60 post        | CDDP+FT      | post WT | c280 | 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.
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 \((P=0.0492,\text{ data not shown})\). 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 \((P=0.0060;\text{ Fig. 3})\). 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.,\(^{3}\) 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

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**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.
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, but reports from other groups had found no such correlation. With regard to esophageal cancers, the relationship of p53 status to prognosis also remains controversial. 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. 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. 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. These data suggest that different mutations in vitro 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. Furthermore, cells with mutated p53 genes often become resistant to such therapies. 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. 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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