Esophageal squamous cell carcinomas if with CDC25B positivity, CRT can be expected. Expressions respond well to CRT. Even with p53 positivity, with negative p53, positive CDC25B, and negative MT expression were found to be significantly more sensitive to chemoradiotherapy (CRT), but some cases are not. Using a retrospective analysis, we aimed to identify the predictors of the response by esophageal squamous cell carcinoma to definitive CRT.

The esophageal squamous cell carcinomas are generally sensitive to chemoradiotherapy (CRT), but some cases are not. Using a retrospective analysis, we aimed to identify the predictors of the response by esophageal squamous cell carcinoma to definitive CRT.

AIM: Esophageal squamous cell carcinoma is generally sensitive to chemoradiotherapy (CRT), but some cases are not. Using a retrospective analysis, we aimed to identify the predictors of the response by esophageal squamous cell carcinoma to definitive CRT.

METHODS: The intensities of expression of p53, Ki67, Bcl-2, Bax, cyclin D1, VEGF, CDC25B, and metallothionein (MT) were evaluated immunohistochemically in the biopsy specimens obtained before CRT, and the intensities of their expression were tested for correlations with the clinical effects of CRT.

RESULTS: The esophageal squamous cell carcinomas with negative p53, positive CDC25B, and negative MT expression were found to be significantly more sensitive to CRT. In addition, p53 positivity and CDC25B positivity respond well to CRT.

CONCLUSION: Esophageal squamous cell carcinomas with negative p53, positive CDC25B, and negative MT expressions respond well to CRT. Even with p53 positivity, if with CDC25B positivity, CRT can be expected.

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Keywords: p53; CDC25B; Metallothionein; Chemoradiotherapy; Esophageal squamous cell carcinomas

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Thirty-six patients with advanced squamous cell carcinoma of the esophagus, who refused surgery and gave informed consent to CRT at Ibaraki Prefectural Central between 1996 and 2001, were included in this study (Table 1). The diagnosis of squamous cell carcinoma was confirmed by histological examination of biopsy specimens obtained before starting CRT (the clinicopathological data are summarized in Table 1). Response to CRT was evaluated clinically after two courses and the evaluation included a barium esophaogram, esophagoscopy, and computed tomography (CT) of the chest and abdomen. A complete response was defined as no visible tumor by esophagoscopy, biopsy specimens free of tumor tissue, and normal CT findings; a partial response as >50% reduction of tumor size; and progressive disease as increasing tumor growth indicated by barium swallow or esophagoscopy and increasing tumor diameter assessed by CT.

Table 1 Characteristics of patients (mean±SD, n)

| Parameters        | Values       |
|-------------------|--------------|
| Sex (male/female) | 33/3         |
| Age (range)       | 63.3±9.2 (42-78 yr) |
| Histopathology    |              |
| Well-differentiated| 6            |
| Moderately differentiated | 23          |
| Poorly differentiated| 7          |
| Stage (UICC)      |              |
| I                  | 4            |
| II                 | 7            |
| III                | 18           |
| IVa                | 7            |

Chemoradiotherapy
Chemotherapy consisted of protracted infusion of 5-FU at a dose of 400 mg/m² per d on d 1-5 and 8-12, combined with a 2-h infusion of CDDP at 40 mg/m² per d on d 1 and 8, repeated twice every 5 wk. Concurrent radiotherapy was started on d 1 at 2 Gy/d for 5 d/wk. The total radiation dose was 60 Gy, with a 2-wk break after a dose of 30 Gy. The patients were followed up every 3 mo for the first 3 years after the end of treatment, and afterward every 6 mo thereafter. New chemotherapy agent (e.g., docetaxel) was applied for the patient with non-effective CRT.

Immunohistological staining and its evaluation
Immunostainings for p53, Ki67, Bcl-2, Bax, cyclin D1, VEGF, MT, and CDC25B were performed using streptavidin-peroxidase complex methods with an EnVision™ peroxidase kit (Dako, Glostrup, Denmark) on a TeckMate Horizon automated staining system (Dako, Glostrup, Denmark). Primary antibodies were incubated overnight at 4 °C with E9 (dilution 1:50; Dako, Kyoto, Japan) for MT, and for 1 h at room temperature with D0-7 (dilution 1:50; Novocastra Laboratories, Newcastle upon Tyne, UK) for p53, with Ki-SS (dilution 1:50; Dako, Kyoto, Japan) for Ki-67, with 124 (dilution 1:50; Dako, Kyoto, Japan) for Bcl-2, with A3533 (dilution 1:50; Dako, Kyoto, Japan) for Bax, with DSC-6 (dilution 1:200; Novocastra) for cyclin D1, with JH 121 (dilution 1:50; Upstate, Lake Placid, NY, USA) for VEGF, and with C45820 (5 μg/mL; Transduction Laboratories, Lexington, KY, USA) for CD25B. The expressions of p53, Bcl-2, Bax, VEGF, cyclin D1, MT, and CD25B were investigated in consecutive histological sections prepared from the biopsy specimens. After being pretreated thrice, each for 5 min, in a citrate buffer (pH 6.0) at 750 W, the slides were separately incubated overnight at 4 °C with the mAbs, and the expressions of p53, Ki-67, Bcl-2, Bax, VEGF, cyclin D1, MT, and CD25B were assessed under light microscope by one observer (Itabashi), who was unknown about the clinical outcome. The percentages of positive tumor cells were determined semiquantitatively, and each sample was assigned to one of the following categories: negative (0-10%) and positive (11-100%). In addition, the intensities of immunostaining of antigens localized in the cytoplasm (Bcl-2, Bax, cyclin D1, VEGF, MT, and CD25B) were classified as negative or positive. Staining intensity was not determined for p53 (nuclear immunostaining), because no significant differences in staining intensity were observed in the p53-positive cases.

Statistical analysis
Associations between two parameters were analyzed by the Spearman’s rank correlation test. For continuous parameters, the difference between the two groups was analyzed by Student’s t test. Determination of the distribution of immunohistochcmical staining between groups was analyzed using χ² analysis. Cumulative survival of the patients was calculated on July 31, 2002, by the Kaplan-Meier method and the statistical significance was analyzed by the log-rank test. P<0.05 was considered statistically significant in all analyses. All statistical analyses were performed using the StatView version 5.0 software package (Abacus Concepts, Berkeley, CA, USA).

The overall proportions of the cells positive for expression of p53, Bcl-2, Bax, Ki67, cyclin D1, VEGF, MT, and CD25B were 58.3%, 27.8%, 30.6%, 86.1%, 41.7%, 58.3%, 27.8%, and 13.9%, respectively (Table 2 and 3). Not MT negativity, but CD25B positivity with p53 positivity is a predictor of a response to CRT (respectively p=0.39, 0.027).The median survival time of the patients with p53-negative and p53-positive patients were 588 and 415 d, respectively (P<0.001, Student’s t-test), but no significant difference was found between the survival curves of the p53-negative and p53-positive patients and esophageal cancer staging (Figures 1 and 2). However, there was a significant association between p53-negative and MT-negative patients and effect of CRT (Table 4). At the end of the follow-up period on July 31, 2002, 25% patients (9/36) were still alive. The follow-up time for all 36 patients ranged from 26.7 to 81 mo (median, 59.5±18.1 mo). In the group treated by CRT, there was a marked difference in mean survival time between the patients with p53-negative tumors (19.6±10.3 mo) and with p53-positive tumors (13.8±8.6 mo). Esophageal squamous cell carcinomas relapsed in
Esophageal cancer is a relatively uncommon but aggressive disease. Surgical resection has been widely accepted as the standard treatment for esophageal cancer, and techniques have improved during the past decades. However, long-term survival after resection of carcinoma of the thoracic esophagus is generally poor, with rates of only 20-42.4%[15-18]. Some reports on CRT have indicated that it offers various advantages in managing esophageal cancer[19]. Oncologists have advocated a non-surgical approach with definitive CRT as the standard treatment for this disease[20-23]. The value of CRT for the treatment of unrespectable esophageal squamous cell carcinoma remains a matter of controversy. Only a few clinical studies have been published since the 1980s, and most patients in those studies had local-regional disease (UICC stage I or II). Several investigators have reported successful results with these modalities, either with or without surgery, against local-regional carcinoma[24-26]. The combination of 5-FU and CDDP has become the standard regimen, not only because of the clinical outcome but also because of the synergism between the two agents and their radiosensitizing effects [27,28]. Recently published results on CRT have indicated that it offers various advantages for the treatment of carcinoma of the esophagus[29,30]. A multicenter study on the indications for CRT as curative therapy for patients with locally advanced disease has suggested that concurrent CRT is potentially curative, even in cases of locally advanced carcinoma of the esophagus (i.e., T4 and/or M1 lymph node metastasis disease)[31]. Some studies have implicated various molecules, including p53, CDC25B, and MT, as candidates for biological markers for the response of human esophageal cancer to CRT[10,14]. We evaluated the role of cell-cycle-regulating molecules in the sensitivity of human squamous cell carcinoma of the esophagus to CRT by immunohistochemical methods and found p53, MT, and CDC25B to be significant independent markers for predicting sensitivity to CRT. Our results showed that negative

### Table 2: Relation between immunohistochemical expressions of biological markers and response of esophageal squamous cell carcinoma to CRT (n)

| Markers     | Responder (CR+PR) | Non-responder (NC+PD) | P    |
|-------------|-------------------|-----------------------|------|
| p53(-)      | 14                | 1                     | 0.0095|
| p53(+)      | 11                | 10                    |      |
| MT (-)      | 21                | 5                     | 0.019 |
| MT (+)      | 4                 | 6                     |      |
| CDC25B(-)   | 7                 | 8                     | 0.0129|
| CDC25B(+)   | 18                | 3                     |      |

Spearman’s rank correlation test.

### Table 3: Relation between expressions of biological markers and response of esophageal squamous cell carcinoma to CRT (n)

| Markers     | Responder (CR+PR) | Non-responder (NC+PD) | P   |
|-------------|-------------------|-----------------------|-----|
| Bax (-)     | 17                | 8                     | 0.799 |
| Bax (+)     | 8                 | 3                     | 0.799 |
| Bcl-2 (-)   | 18                | 8                     | 0.806 |
| Bcl-2 (+)   | 7                 | 3                     | 0.806 |
| Cyclin D1(-)| 15                | 6                     | 0.305 |
| Cyclin D1(+)| 10                | 5                     | 0.305 |
| VEGF (-)    | 10                | 5                     | 0.936 |
| VEGF (+)    | 15                | 6                     | 0.936 |
| Ki67 (-)    | 4                 | 1                     | 0.586 |
| Ki67 (+)    | 21                | 10                    | 0.586 |

Spearman’s rank correlation test.

### Table 4: Relation between effects of CRT and immunohistochemical staining

| Immunoreactivity/response     | P     |
|------------------------------|-------|
| p53 -                        |       |
| CDC25B +                     |       |
| Responders (CR+PR)           |       |
| 0.027                        |       |
| Non-responder (NC+PD)        |       |
| MT -                         |       |
| 0.0305                       |       |
| CDC25B +                     |       |
| Responders (CR+PR)           |       |
| 21                            |       |
| Non-responder (NC+PD)        |       |
| 2                             |       |
| 5                             |       |
| 2                             |       |

χ² test.
immunostaining for p53 in pre-CRT biopsy specimens predicted a good response. In addition to being the only established biological marker for response to CRT in clinical studies, p53 is the best characterized and most powerful marker[6,23], and it is commonly acknowledged to be a definite indicator of radiation sensitivity in various cancers[24,31]. The p53 immunoreactivity is generally thought to be attributable to the accumulation of abnormal p53 protein[24]. In our results, even the tumors with p53 positivity were sensitive to the CRT. In most of the esophageal squamous cell carcinoma, p53 are positive immunohistologically. Even with p53 CRT sensitivity is sure to exist. G2-M checkpoint, in which the pathway. Among the various molecules that regulate G2-M checkpoint, in which pathway are regulated by G2-M checkpoint. In our results, even the tumors with p53-negative and p53-positive patients. It might be because a new chemotherapeutic agent (e.g., docetaxel) was applied for the patients with non-effective CRT. Docetaxel is an active drug for treating squamous cell carcinoma of head and neck. For the patients with recurrent or metastatic disease of squamous cell carcinoma, docetaxel could be used as a second line chemotherapy[80]. From a clinical standpoint, it is very important that a prospective study based on this result should be done. There are only retrospective studies that have been reported up to now. Standard therapy may be developed from the results of a prospective study based on the immunohistochemical characteristics of the tumors of the biopsy.

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