Murine Double Minute 2 and Its Association with Chemoradioresistance of Esophageal Squamous Cell Carcinoma

HIROSHI OKAMOTO[1,3], FUMIYOSHI FUJISHIMA[3], YASUHIRO NAKAMURA[2], MASASHI ZUGUCHI[1], GO MIYATA[1], TAKASHI KAMEI[1], TORU NAKANO[1], KAZUNORI KATSURA[1], SHIGEO ABE[1], YUSUKE TANIYAMA[1], JIN TESHIMA[1], MIKA WATANABE[3], AKIRA SATO[1], NORIAKI OHUCHI[1] and HIRONOBU SASANO[2,3]

[1]Division of Advanced Surgical Science and Technology and [2]Department of Pathology, Graduate School of Medicine, Tohoku University, Aoba-ku, Sendai, Miyagi, Japan; [3]Department of Pathology, Tohoku University Hospital, Aoba-ku, Sendai, Miyagi, Japan

Abstract. *Background: Definitive chemoradiotherapy (dCRT) has been established as the standard treatment for esophageal squamous cell carcinoma (ESCC). However, many patients develop persistent or recurrent disease following dCRT. We investigated factors related to chemoradioresistance and treatment outcomes in patients with ESCC who underwent salvage esophagectomy after dCRT. Patients and Methods: We selected 38 patients with persistent disease and 24 with recurrent disease who underwent salvage esophagectomy after dCRT, immunolocalized p53, p16, p27, murine double minute 2 (MDM2), cyclin D1, Ki-67, and epidermal growth factor receptor, and correlated the findings with clinicopathological features. Results: MDM2 positivity was significantly higher among patients with persistent disease than among those with recurrent disease ($p<0.0001$). In addition, negative p16 expression was a predictor of poor prognosis among patients with persistent disease. Conclusion: MDM2 overexpression plays an important role in chemoradioresistance of ESCC; furthermore, negative p16 expression can predict poor prognosis of patients with persistent disease.*

Definitive chemoradiotherapy (dCRT) has become the standard treatment for esophageal carcinoma (1-3), and results of some previous studies, including our own, have shown comparable clinical outcomes between patients undergoing dCRT and those undergoing surgery alone (4, 5). However, it is also true that 34.2%-56.0% of patients who undergo dCRT experience persistent or recurrent disease (2, 4-6), which generally results in adverse clinical outcomes. We have aggressively performed salvage esophagectomy after dCRT since October 2001 to improve the chances of survival among these patients (6-9). However, patients who undergo salvage esophagectomy after dCRT still exhibit a high rate of morbidity and mortality (6, 7, 9). If we could determine the chemoradiosensitivity of disease in such patients at the time of diagnosis, we could dramatically improve treatment strategies and clinical outcomes. In addition, if cases with a poor prognosis after salvage esophagectomy could be identified at an earlier clinical stage, much more stringent follow-up and administration of more aggressive adjuvant therapy could also confer clinical benefits to these patients.

Salvage esophagectomy is a rather restricted procedure because of its high-risk nature. To the best of our knowledge, a detailed evaluation of surgical pathology specimens obtained during salvage esophagectomy, following dCRT, has not been previously reported. Therefore, in this study, we retrospectively evaluated the clinicopathological and immunohistochemical features of esophageal squamous cell carcinoma (ESCC) specimens obtained from patients who underwent salvage esophagectomy after dCRT in order to explore the factors related to chemoradioresistance among patients with ESCC. We immunolocalized p53, p16, p27, murine double minute 2 (MDM2), cyclin D1, Ki-67, and epidermal growth factor receptor (EGFR) because all of these are known prognostic factors for ESCC and/or are reportedly related to chemoradioresistance (10-16).

Correspondence to: Hiroshi Okamoto, MD, Division of Advanced Surgical Science and Technology, Graduate School of Medicine, Tohoku University, 1-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8574, Japan. Tel: +81 227177214, Fax: +81 227177217, e-mail: hi-ok-0531@med.tohoku.ac.jp

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Patients and Methods

Patients. We performed 68 salvage esophagectomies following dCRT at the Tohoku University Hospital (Sendai, Japan) between September 2001 and November 2008. Two cases of distant metastasis, three cases of initial endoscopic treatment, and one case of previous CRT for head and neck cancer were excluded. Therefore, 62 patients with ESCC were included in the study, out of whom 38 had persistent disease and 24 had recurrent disease. The definitions of persistent and recurrent disease used in this study are described in the next section.

dCRT and salvage esophagectomy. The dCRT protocol in our study basically followed that of the Japan Clinical Oncology Group (JCOG) trial 9906 (2). In brief, the protocol comprised of two cycles of intravenous cisplatin (40 mg/m^2) infusion on days 1 and 8 and continuous 5-fluorouracil (400 mg/m^2) infusion over 24 h on days 1-5 and 8-12 every five weeks with concurrent radiotherapy (60 Gy administered in 30 fractions over a period of eight weeks, including a 2-week rest period after the administration of 30 Gy). The radiotherapy administered is three-dimensional. Gross tumour volume (GTV) included the primary tumour and metastatic lymph nodes evaluated by endoscopy, computed tomography (CT), and 2-[^{18}F]fluoro-2-deoxy-D-glucose positron-emission tomography (FDG-PET), if necessary. The clinical target volume included the GTV and the supraclavicular, mediastinal, and celiac axis lymph node regions. When the tumours were located in the upper third of the esophagus, the celiac axis lymph node region was excluded. After 40 Gy, an extra boost of radiation was administered to GTV *via* an oblique approach (20 Gy administered in 10 fractions). Additional chemotherapy was administered until May 2004. This comprised of two cycles of 80 mg/m^2 of cisplatin on day 1 and a continuous infusion of 800 mg/m^2 of 5-fluorouracil on days 1-5, every four weeks. Clinical evaluation by endoscopy with biopsy, CT, and FDG-PET (if necessary) were performed one month after treatment completion. Patients who were evaluated as having an incomplete response at this time and who subsequently underwent salvage esophagectomy were defined as persistent cases. Patients who were evaluated as having a complete response (CR) at this time, but whose tumours subsequently recurred in the same location and who underwent salvage esophagectomy later were defined as recurrent cases. Recurrence was confirmed by biopsy. A total of 24 out of the 38 patients with persistent disease and 15 out of the 24 patients with recurrent disease underwent the JCOG 9906 protocol. The other patients underwent treatment according to different protocols followed by other hospitals or other Departments (Radiation Oncology, Clinical Oncology, or Otolaryngology) of the Tohoku University Hospital. The treatments that the patients received are summarized in Table I. Some patients required a dose decrease or a delay in chemotherapy because of adverse side-effects. All patients received the complete scheduled radiation dose.

Salvage esophagectomy was usually performed under thoracoscopic guidance. One- or two-field lymph node dissection was performed for tumours of the cervical esophagus or the middle or lower thirds of the esophagus, and 3-field lymph node dissection was performed for tumours of the upper third of the esophagus.

Immunohistochemical staining and pathological evaluation. Surgical specimens were fixed in 10% formalin and representative sections were embedded in paraffin wax. Excluding specimens in which no residual tumour cells were observed microscopically, the specimens of 33 patients with persistent disease and 22 patients with recurrent disease were evaluated immunohistochemically. Immunohistochemical staining was performed using the streptavidin–biotin complex method, as follows. Serial 4-μm-thick sections from the most representative area of each specimen were de-paraffinized in xylene, rehydrated in a graded ethanol series, and then immersed in 3.0% hydrogen peroxide in methanol for 10 min at room temperature (RT) to block endogenous peroxidase activity. For antigen retrieval, the slides for p53 were heated in a microwave at 95˚C for 15 min in 0.01 M citrate buffer (pH 6.0). The slides for p16, p27, MDM2, cyclin D1, and Ki-67 were heated for 5 min in 0.01 M citrate buffer (pH 6.0) using an autoclave at 121˚C. The slides for EGFR were incubated in 0.05% protease in Tris-HCL buffer (pH 7.6) at 37˚C for 10 min. The slides were incubated in 1% normal rabbit (for mouse monoclonal antibody) or goat (for rabbit monoclonal antibody) serum for 30 min at RT to reduce non-specific antibody binding. Subsequently, the slides were incubated at 4˚C overnight with mouse monoclonal antibody against p53 (DO-7, diluted 1/100; Nichirei Biosciences Inc., Tokyo, Japan), p16 (G175-1239, diluted 1/100; BD Biosciences, Franklin Lakes, NJ, USA), p27 (SX53G8, diluted 1/800; Dako, Glostrup, Denmark), MDM2 (SMP14 diluted 1/1000; Santa Cruz Biotechnology Inc., CA, USA), Ki-67 (MIB-1, diluted 1/300; Dako), EGFR (31G7, used as delivered, product code 413701; Nichirei Biosciences Inc.), and rabbit monoclonal antibody against cyclin D1 (SP4, used as delivered, product code 413521; Nichirei Biosciences Inc.). The next day, the sections were incubated with biotinylated anti-mouse or anti-rabbit immunoglobulin (Nichirei Biosciences Inc.) as secondary antibodies and incubated with peroxidase-labeled streptavidin (Nichirei Biosciences Inc.) for 30 min at RT. The antigen–antibody complexes were visualized with 3,3'-diaminobenzidine, and the slides were counterstained with Mayer's haematoxylin, dehydrated in a graded ethanol series, and cleared in xylene.

The staining and pathological findings were evaluated independently by two of the authors (HO and FF) who were blinded to the patients' clinical data. The histopathological findings were classified according to the seventh edition of the Union for International Cancer Control system (17). The percentage of p53-, p27-, MDM2-, Ki-67-, and cyclinD1-positive nuclei was determined for more than three regions of the deepest area of the tumour and 1000 viable tumour cells were evaluated at a magnification of ×400 by microscopy. For p16, the percentage of cells with positive nuclei and positive cytoplasm was determined, and for EGFR, the percentage of cells with positive membranes was determined. The cutoff values for abnormal expression were as follows: p53, ≥10% (13); p16, ≤5% (16); p27, ≥10% (15); MDM2, ≥20% (18); cyclin D1, ≥10% (14); Ki-67, ≥39% (12). Scoring for EGFR was performed using the immunoreactive score (IRS) obtained by multiplying the intensity score (0=no staining, 1=faint staining, 2=moderate staining, 3=strong staining) by the extent score (0=none, 1=<10%, 2=10%-50%, 3=>50%-80%, 4=>80%), and ranged from 1 to 12. It was decided that an IRS ≥6 was indicative of abnormal expression (10). Histopathological tumour regression was classified into five categories according to the Japanese Classification of Esophageal Cancer, tenth edition (19) as follows: grade 3, markedly effective (no viable residual tumour cells); grade 2, moderately effective (less than one-third residual tumour cells); grade 1, slightly effective (1b, one-third to two-thirds residual tumour cells; 1a, more than two-thirds residual tumour cells); grade 0, ineffective (no therapeutic effect observed).

Table I. *Summary of treatments*.

Patients with persistent disease	n=38
JCOG9906 protocol	24 (63.2%)
Cisplatin/5-FU/50 Gy	1 (2.6%)
Cisplatin/5-FU/60 Gy	1 (2.6%)
Cisplatin/5-FU/64 Gy	1 (2.6%)
Nedaplatin/5-FU/60 Gy	2 (5.3%)
Nedaplatin/5-FU/64 Gy	2 (5.3%)
Nedaplatin/5-FU/70 Gy	6 (15.8%)
Cisplatin/5-FU/DOC/70 Gy	1 (2.6%)
Patients with recurrent disease	**n=24**
JCOG9906 protocol	15 (62.5%)
Cisplatin/5-FU/60 Gy	2 (8.3%)
Cisplatin/5-FU/64 Gy	1 (4.2%)
Cisplatin/5-FU/70 Gy	1 (4.2%)
Nedaplatin/5-FU/60 Gy	2 (8.3%)
Nedaplatin/5-FU/69.6 Gy	1 (4.2%)
Nedaplatin/5-FU/70 Gy	1 (4.2%)
Nedaplatin/DOC/68.4 Gy	1 (4.2%)

JCOG, Japan Clinical Oncology Group; 5-FU, 5-fluorouracil; DOC, docetaxel.

Statistical analysis. Continuous data were analysed using the Student's *t*-test or the Mann–Whitney *U*-test. Categorical data were evaluated using Pearson's chi-square test, Fisher's exact test, or the Mann–Whitney *U*-test as appropriate. Normality was assessed using the Shapiro–Wilk test. Equality of variances was evaluated using the F test. Overall curves were determined by the Kaplan–Meier method, and a log-rank test was used to compare the survival curves. The patient survival time was determined from the date of salvage surgery until death or the last follow-up examination. All statistical analyses were performed using JMP Pro Version 9.0.2 (SAS Institute Inc., Cary, NC, USA). Two-tailed *p*-values <0.05 were considered statistically significant.

This study was approved by the Ethical Committee of Tohoku University Hospital (accession number 2011-596).

Results

Comparison of clinicopathological features and survival outcomes between patients with persistent and recurrent disease. The median follow-up time for patients with persistent and recurrent disease was 12.5 months (range=0-102 months) and 34.5 months (range=4-102 months), respectively. Twelve out of 38 patients with persistent disease and three out of 24 patients with recurrent disease underwent non-curative resection (R1/R2). The clinicopathological features of the patients with persistent and recurrent disease are shown in Table II. Pathological tumour depth, lymph node status, and tumour stage were significantly more advanced among patients with persistent disease than among those with recurrent disease. In terms of tumour differentiation, poorly-differentiated tumours were more frequently observed in patients with recurrent disease than in those with persistent disease. The 3- and 5-year overall

Table II. *Clinicopathological features of patients with persistent and recurrent disease*.

Variable	Persistent disease (n=33) (%)	Recurrent disease (n=22) (%)	*p*-Value
Mean age±SD (Range), years	63.9±7.8 (51-80)	65.6±9.1 (44-82)	0.28
Gender			
Male	28 (84.8)	20 (90.9)	0.69
Female	5 (15.2)	2 (9.1)	
Location			
Cervix	0 (0.0)	3 (13.6)	0.14
Upper	4 (12.1)	1 (4.5)	
Middle	19 (57.6)	10 (45.5)	
Lower	10 (30.3)	8 (36.4)	
Radiation			
60 Gy	23 (69.7)	16 (72.7)	0.81
>60 Gy	10 (30.3)	6 (27.3)	
Additional chemotherapy			
Not performed	28 (84.8)	15 (68.2)	0.19
Performed	5 (15.2)	7 (31.8)	
Histological type			
Well differentiated	3 (9.1)	1 (4.5)	0.06
Moderately differentiated	27 (81.8)	13 (59.1)	
Poorly differentiated	3 (9.1)	8 (36.4)	
pT			
1	1 (3.0)	8 (36.4)	0.0005
2	6 (18.2)	4 (18.2)	
3	23 (69.7)	5 (22.7)	
4	3 (9.1)	5 (22.7)	
pN			
0	13 (39.4)	18 (81.8)	0.0019
1-3	20 (60.6)	4 (18.2)	
pStage			
I	4 (12.1)	11 (50.0)	0.015
II	10 (30.3)	5 (22.7)	
III	17 (51.5)	6 (27.3)	
VI	2 (6.1)	0 (0.0)	
Lymphatic invasion			
Negative	17 (51.5)	12 (54.5)	0.83
Positive	16 (48.5)	10 (45.5)	
Venous invasion			
Negative	7 (21.2)	8 (36.4)	0.22
Positive	26 (78.8)	14 (63.6)	
Residual tumour			
R0	21 (63.6)	19 (86.4)	0.064
R1/R2	12 (36.4)	3 (13.6)	
Tumour regression grade			
0	1 (3.0)		
1a	20 (60.6)		
1b	9 (27.3)		
2	3 (9.1)		

SD, Standard deviation.

survival (OS) rates for all 62 patients were 35.1% and 28.0%, respectively. The survival outcomes of patients with persistent and recurrent disease are compared in Figure 1. The 3- and 5-year OS rates were 28.2% and 20.6%, respectively, for patients with persistent disease and 45.8% and 41.3%, respectively, for patients with recurrent disease. The OS rate of patients with persistent disease was significantly worse than that of patients with recurrent disease (p=0.044).

Comparison of marker expression between patients with persistent and recurrent disease. Marker expression among the patients with persistent and recurrent disease is summarized in Figure 2. The MDM2 positivity rate (p<0.0001) and the IRS for EGFR (p=0.030) were significantly higher among patients with persistent disease than among those with recurrent disease. On the other hand, the Ki-67 positivity rate tended to be higher among patients with recurrent disease than among those with persistent disease (p=0.062). None of the other markers exhibited any significant correlations with persistent or recurrent disease. Tumour cells positive for MDM2, p16, Ki-67, and EGFR expression are illustrated in Figure 3.

Correlations between marker expression and clinicopathological features. Among the patients with persistent disease, EGFR expression was correlated with advanced pathological stage (p=0.036, data not shown) and lymphatic invasion (p=0.024, data not shown). No other significant correlations were observed.

Survival analysis of clinicopathological findings and marker expression among patients with persistent and recurrent disease. Among patients with persistent disease, survival analysis showed that pathological tumour depth, pathological stage, lymphatic invasion, residual tumour, and p16 status were significant prognostic factors for OS (Table III and Figure 4). Among patients with recurrent disease, pathological tumour depth, pathological stage, lymphatic invasion, and residual tumour were significant prognostic factors for OS (Table III).

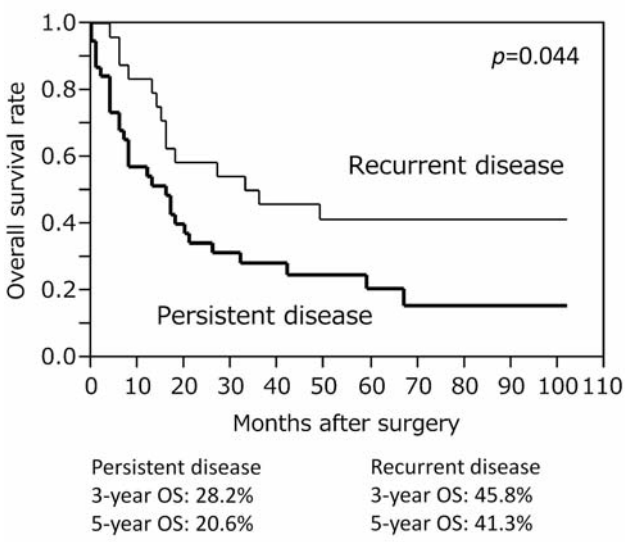

Figure 1. *Comparison of survival outcomes between patients with persistent and recurrent disease. Overall survival (OS) was significantly worse among patients with persistent disease than among those with recurrent disease (p=0.044).*

Discussion

The oncoprotein MDM2 inhibits p53 by directly blocking its transcriptional activity or ubiquitinating p53 to promote p53 resolution in cytoplasmic proteasomes (20, 21). MDM2 overexpression induced by ionizing radiation inhibits mediation of cell-cycle arrest in the G_1 phase and apoptosis by p53 (22, 23), which may explain why some tumours resist radiotherapy or CRT. On the other hand, Ki-67 is a widely known marker of cell proliferation. In this study, tumour MDM2 positivity was significantly higher among patients with persistent disease than among those with recurrent disease. In addition, Ki-67 positivity tended to be higher among those with recurrent disease. Therefore, the biological behaviour of persistent disease appears to differ from that of recurrent disease with regard to resistance to chemoradiation. Persistent disease is usually characterized by marked chemoradioresistance, whereas recurrent disease occurs in patients who have once been evaluated as having a complete clinical response. Considering that tumours with increased resistant to CRT are more frequently identified as persistent rather than recurrent, high levels of MDM2 seem to play a critical role in chemoradioresistance of ESCC cells. Ikeguchi *et al*. (11) reported that the correlation between MDM2 expression in ESCC and shorter survival was more marked for patients who underwent postoperative adjuvant CRT than for those who did not. These results together with those in our present study clearly indicate that MDM2 expression in ESCCs that display chemoradioresistance is already high before treatment and remains stable or increases after CRT. If this observation is valid, we may be able to determine chemoradiosensitivity in patients with ESCC by examining MDM2 expression in biopsy specimens obtained before treatment, or by examining the increase in MDM2 positivity in biopsy specimens obtained after induction CRT; however, this awaits further investigations for clarification. Recently, the effects of the MDM2 inhibitor Nutlin-3 (24) were clinically evaluated, especially with regard to the treatment of leukemia (25, 26). Nutlin-3 inhibits MDM2 and causes cell-cycle arrest, apoptosis, and senescence through the

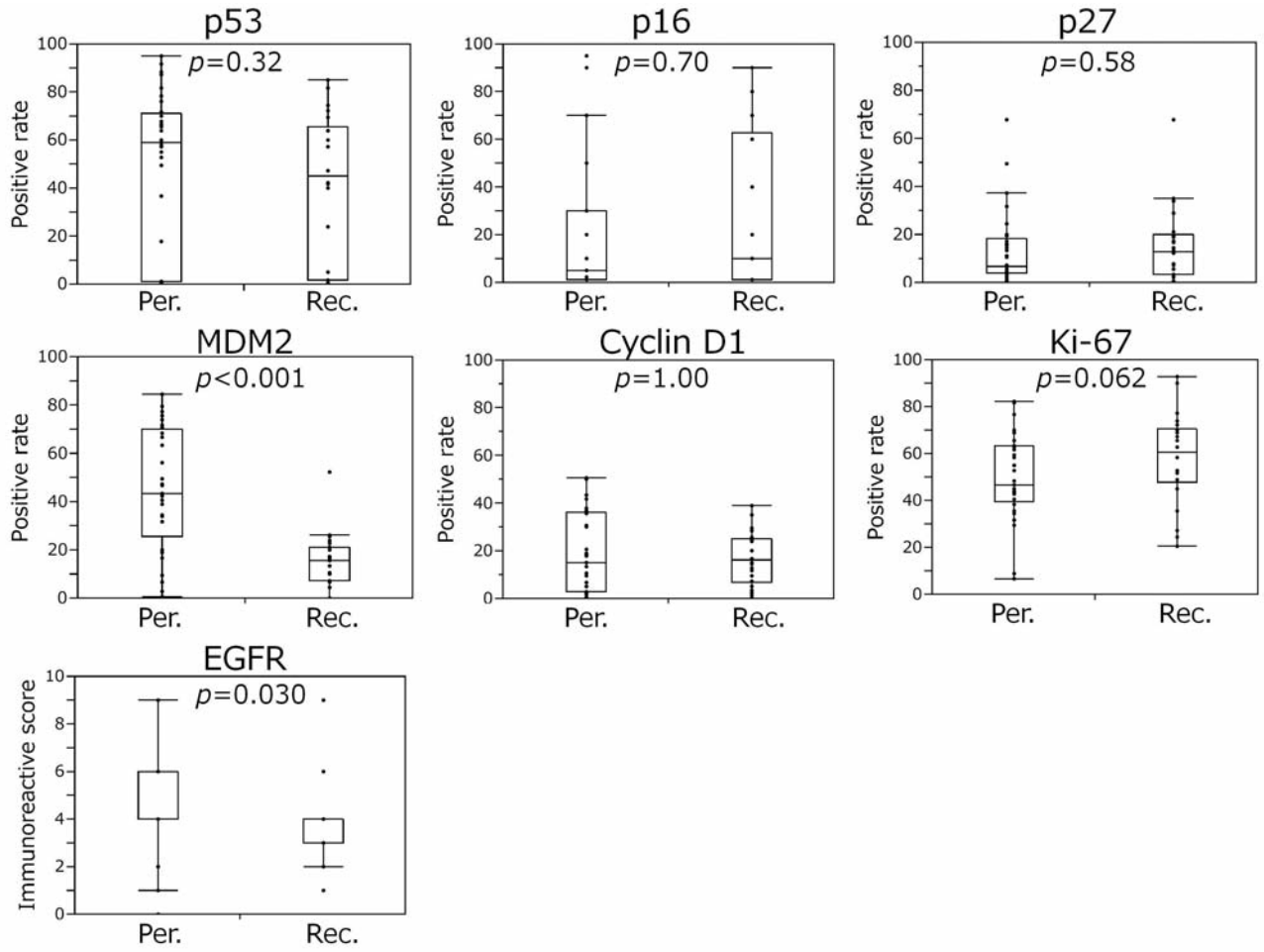

Figure 2. *Comparison of marker expression between patients with persistent and recurrent disease. Murine double minute 2 (MDM2) positivity rate (p<0.0001) and the immunoreactive score of epidermal growth factor receptor (EGFR) (p=0.030) were significantly higher among patients with persistent disease than among those with recurrent disease. On the other hand, the Ki-67 positivity rate tended to be higher among patients with recurrent disease than among those with persistent disease (p=0.062). Per., Persistent disease; Rec., recurrent disease.*

accumulation and activation of p53. Arya *et al*. (27) reported that Nutlin-3 improved the radiosensitivity of laryngeal squamous carcinoma cells. Therefore, CRT in conjunction with Nutlin-3 may contribute to an improvement in response rate among patients with ESCC.

It appears that recurrent tumour cells have good chemoradiosensitivity but remain in the esophageal wall and regrow in the same location of the esophagus as that of the primary tumour. Considering that the tumours of patients with recurrent disease tend to exhibit high Ki-67 expression, these tumours seem to have a high proliferative capacity. Patients evaluated as having a clinical complete response included those in whom carcinoma cells actually remained in the esophageal wall. Therefore, early detection of recurrence may lead to an improved prognosis. When early-stage recurrence is found, a good prognosis can be expected after salvage esophagectomy, as shown in this study. Moreover, endoscopic treatment can be considered to preserve the esophagus.

Regarding EGFR, IRS was significantly higher in patients with persistent disease than in patients with recurrent disease. This may be because the pathological stage of tumours was significantly more advanced in patients with persistent disease than in those with recurrent disease and because EGFR expression was correlated with advanced pathological stage among patients with persistent disease.

p16 is a cyclin-dependent kinase inhibitor (28). Inactivation of p16 has been observed in several human malignancies, including ESCC (29, 30). In this study, p16 expression was significantly correlated with the survival of patients with persistent disease. Although R0 resection contributes to survival after salvage esophagectomy (7, 9), this finding suggests that patients who undergo salvage

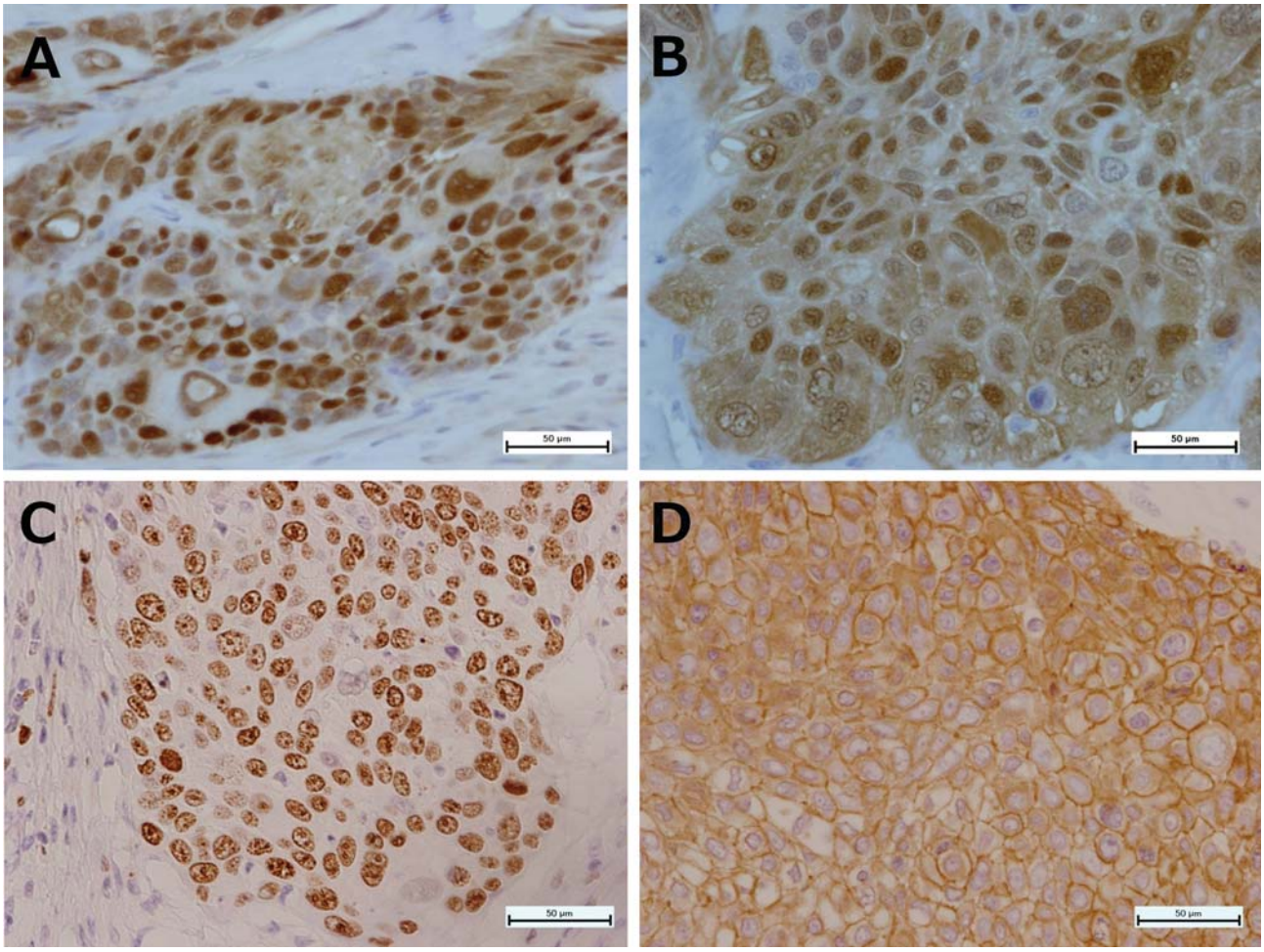

Figure 3. *Immunohistochemical staining of esophageal squamous cell carcinoma Tumour cells positive for murine double minute 2 (A), p16 (B), Ki-67 (C), and epidermal growth factor receptor (D) expression (×400 magnification).*

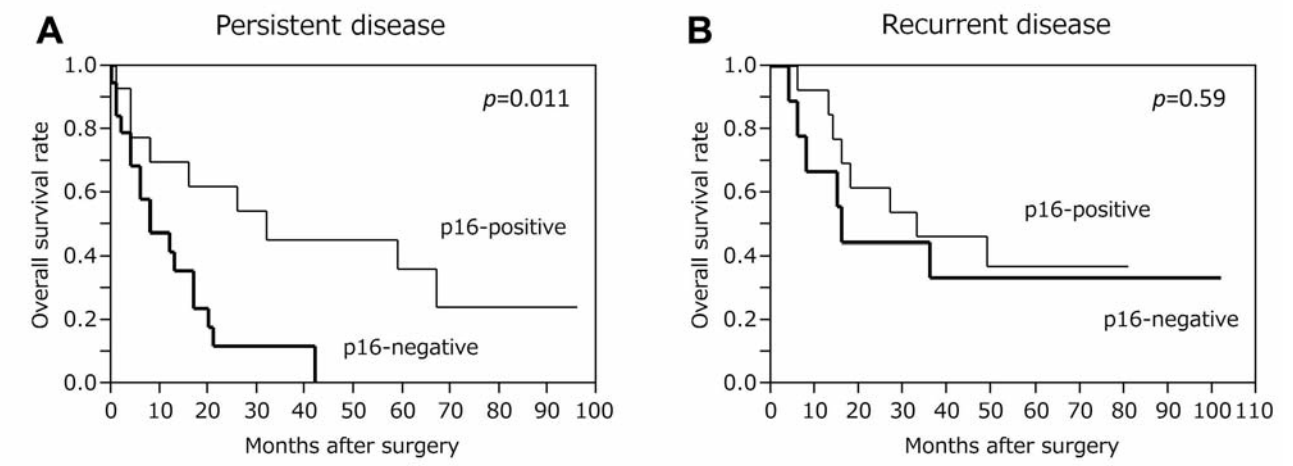

Figure 4. *Kaplan–Meier curves of patients prepared on the basis of p16 expression Among patients with persistent disease, overall survival was significantly shorter in those with negative p16 expression than in those with positive p16 expression (A); however, no statistically significant differences were observed in survival according to p16 status among patients with recurrent disease (B).*

Table III. *Survival analysis of clinicopathological findings and marker expression among patients with persistent and recurrent disease.*

		Persistent disease				Recurrent disease		
Variable	No.	3-Year OS (%)	5-Year OS (%)	*p*-Value	No.	3-Year OS (%)	5-Year OS (%)	*p*-Value
Age (years)								
<60	11	38.4	25.6	0.20	4	25.0	25.0	0.65
≥60	22	19.3	12.9		18	44.4	38.1	
Gender								
Male	28	22.4	18.0	0.39	20	40.0	35.0	0.92
Female	5	50.0	0.0		2	50.0	50.0	
Location								
Cervix/Upper	4	25.0	25.0	0.65	4	25.0	25.0	0.66
Middle/Lower	29	26.3	15.8		18	44.4	38.1	
Radiation								
60 Gy	23	29.4	19.6	0.23	16	37.5	31.3	0.66
>60 Gy	10	30.0	15.0		6	50.0	50.0	
Additional chemotherapy								
Not performed	28	23.6	17.7	0.25	15	40.0	40.0	0.93
Performed	5	40.0	20.0		7	42.9	28.6	
Histological type								
Well/Moderately-differentiated	30	25.4	20.3	0.94	14	42.9	42.9	0.83
Poorly-differentiated	3	33.3	0.0		8	37.5	25.0	
pT								
1/2	7	83.3	55.6	0.0062	12	58.3	48.6	0.046
3/4	26	12.1	8.1		10	20.0	20.0	
pN								
0	13	42.3	31.7	0.096	18	44.4	38.1	0.50
1-3	20	14.8	7.4		4	25.0	25.0	
pStage								
I/II	14	46.8	35.1	0.034	16	56.3	49.2	0.0005
III/VI	19	11.8	5.9		6	0.0	0.0	
Lymphatic invasion								
Negative	17	43.2	34.6	0.019	12	66.7	58.3	0.029
Positive	16	7.3	0.0		10	10.0	10.0	
Venous invasion								
Negative	7	42.9	21.4	0.49	8	50.0	50.0	0.65
Positive	26	21.9	16.4		14	35.7	26.8	
Residual tumour								
R0	21	41.8	27.9	<0.0001	19	47.4	41.5	0.006
R1/2	12	0.0	0.0		3	0.0	0.0	
TRG								
0/1a	21	23.8	14.3	0.31				
1b/2	12	28.5	28.5					
p53								
Negative	9	13.9	13.9	0.50	7	42.9	42.9	0.49
Positive	24	30.0	20.0		15	40.0	32.0	
p16								
Negative	19	11.8	0.0	0.011	9	33.3	33.3	0.59
Positive	14	45.1	36.1		13	46.2	36.9	
p27								
Negative	18	34.2	34.2	0.47	10	30.0	30.0	0.41
Positive	15	20.0	6.7		12	50.0	40.0	
MDM2								
Negative	7	14.3	14.3	0.43	15	33.3	25.0	0.18
Positive	26	30.5	20.3		7	57.1	57.1	
Cyclin D1								
Negative	14	44.5	14.8	0.33	7	28.6	28.6	0.45
Positive	19	15.8	15.8		15	46.7	38.9	
Ki-67								
<39	8	42.9	0.0	0.53	4	25.0	25.0	0.60
≥39	25	21.7	21.7		18	44.4	38.1	
EGFR								
Negative	19	21.2	21.2	0.95	18	38.9	32.4	0.64
Positive	14	33.3	11.1		4	50.0	50.0	

OS, Overall survival; TRG, tumour regression grade; MDM2, murine double minute 2; EGFR, epidermal growth factor receptor.

esophagectomy for tumours with low p16 expression may clinically benefit from stricter perioperative management, aggressive adjuvant therapy, and careful follow-up. In the present study, p16 expression was evaluated only after dCRT. Therefore, the expression of this marker in pretreatment biopsy specimens remains to be evaluated.

A positive correlation between tumour regression grading (TRG) and ESCC prognosis has been reported (13), but no correlation between TRG (0/1a *vs*. 1b/2) and patient survival was detected in this study. The period from dCRT to salvage esophagectomy varied among patients, and only three specimens from patients with persistent disease were TRG2. Moreover, TRG3 specimens were excluded. We believe this to be the reason why TRG was not necessarily correlated with survival in this study. Further investigation of ESCC specimens obtained after neoadjuvant CRT in patients with a fixed interval between chemoradiotherapy and surgery is required.

In conclusion, to the best of our knowledge, this is the first study to undertake a detailed evaluation of surgical pathology specimens obtained during salvage esophagectomy following dCRT. Our findings indicate that overexpression of MDM2 plays an important role in the chemoradioresistance of ESCC cells and that low or lack of p16 expression has the potential to predict poor prognosis among patients with persistent disease after dCRT. We believe that these results and those of further investigations will contribute to the development of a new treatment strategy for ESCC.

Conflicts of Interests

The Authors declare that they have no conflicts of interest.

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