PERGAMON

European Journal of Cancer 36 (2000) 987–993

European Journal of Cancer

www.ejconline.com

Histological response of cisplatin predicts patients' survival in oesophageal cancer and p53 protein accumulation in pretreatment biopsy is associated with cisplatin sensitivity

Y. Shimada [a,*], G. Watanabe [a], S. Yamasaki [a], M. Maeda [a], A. Kawabe [a], J.-I. Kaganoi [a], A. Itami [a], M. Fukumoto [b,c], Y. Kanda [b,d], M. Imamura [a]

[a] *Department of Surgery and Surgical Basic Science, Graduate School of Medicine, Kyoto University, Kawaracho Shogoin Sakyo-ku, Kyoto 606-8507, Japan*
[b] *Department of Pathology and Tumour Biology, Kyoto University, Kyoto 606-8507, Japan*
[c] *Department of Pathology, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan*
[d] *Department of Surgery, Nagahama City Hospital, Nagahama, Japan*

Received 20 September 1999; received in revised form 24 December 1999; accepted 10 February 2000

Abstract

The aim of this study was to evaluate whether cisplatin sensitivity relates to patient's prognosis in oesophageal squamous cell carcinoma and to find a useful chemosensitivity molecular marker. 59 oesophageal squamous cell carcinoma (SCC) patients received cisplatin 30 mg/m^2/week treatment of two to five cycles, followed by oesophagectomy. We analysed retrospectively whether the histological effect was related to patient's prognosis. Furthermore, to evaluate the relationship between the effect of preoperative cisplatin treatment and p53 and cyclin D1 expression, we investigated p53 and cyclin D1 expression in pretreatment biopsy samples using an immunohistochemical analysis and compared the results with the histological effect to cisplatin in the resected oesophagus. The cases that showed immunohistochemical p53 staining in the pretreatment biopsy samples were resistant to cisplatin ($P=0.032$). However, there was no relationship between cyclin D1 expression and histological effect ($P=0.230$). Nevertheless, combined analysis of p53 and cyclin D1 can predict histological effect ($P=0.032$). The prognosis of cisplatin-sensitive cases was significantly better than that of cisplatin-resistant cases ($P=0.041$). Cox's multivariate analysis revealed that the histological effect was an independent prognostic factor. In contrast, p53 protein accumulation and cyclin D1 were not. Histological response after neoadjuvant cisplatin treatment is a prognostic factor for oesophageal SCC and cisplatin chemotherapy may be selected according to the findings of p53 and cyclin D1 expression.

Keywords: p53; Cyclin D1; Oesophageal cancer; Squamous cell carcinoma; Cisplatin sensitivity; Histological response

1. Introduction

Although surgical techniques and perioperative management have progressed, the prognosis for patients with oesophageal cancer remains poor. Recently, preoperative chemotherapy has become one of the treatments of choice in oesophageal squamous cell carcinoma (SCC). However, the clinical effects of preoperative treatment are controversial [1–3]. With regard to chemotherapy in oesophageal SCC, various cisplatin-based neoadjuvant protocols such as cisplatin alone [4], cisplatin + 5-fluorouracil (5-FU) (FP), cisplatin + 5-FU + leucovorin (FLP) and FP + radiation have been proposed. However, there are no definite chemosensitivity markers for cisplatin (even for a single administration regimen) in oesophageal SCC. *TP53* mutation has been proposed as a sensitivity marker for cisplatin-based treatment, but the results are controversial. Ribeiro and colleagues [5] reported that cases showing *TP53* mutation were resistant to concurrent chemoradiotherapy in oesophageal cancer and Muro and associates [6] reported opposite results. In contrast, cyclin D1 has been reported as a prognostic factor of oesophageal SCC [7]. This is a related cell cycle regulator and has also been reported to have a possible effect on cisplatin chemosensitivity [8].

* Corresponding author. Tel.: +81-75-751-3626; fax: +81-75-751-4390.
E-mail address: shimada@kuhp.kyoto-u.ac.jp (Y. Shimada).

0959-8049/00/$ - see front matter
PII: S0959-8049(00)00049-6

Previously, we reported that neoadjuvant low-dose cisplatin administration (30 mg/m^2/week treatment of two cycles) was an effective protocol in advanced oesophageal SCC [4]. Until now, this regimen has been useful and safe, therefore, we planned to find a useful sensitivity marker of this treatment regimen. In this paper, we examined if chemosensitivity relates to patient's prognosis. We also investigated whether there was any relationship between the effect of preoperative cisplatin treatment and either p53 or cyclin D1 expression in oesophageal cancer patients.

2. Patients and methods

2.1. Oesophageal cancer patients

From 1988 to 1997, 91 oesophageal cancer patients (mainly T2, T3, T4 or N1 cases) were treated with low-dose cisplatin (30 mg/m^2/week, two to five cycles), then, the resected oesophagi were examined for histological effects. Clinical effects of cisplatin were diagnosed by computed tomography (CT) scan, magnetic resonance imaging (MRI), ultrasonography, oesophagogastrography and endoscopy. In clinically effective cases which included NC (no change: change in the sum of the products of the diameter of all measured lesions less than 25%) and MR (minor response: tumour size decreased more than 25% but less than 50%), the patients received additional cycles (one to three) administered before the operation according to the clinical response and renal function. An oesophagectomy was performed within 2 weeks after final administration of cisplatin. The standard surgical method, which was used, has been previously described [4,9]. In brief, oesophagectomy with lymph node dissection was performed by means of a right thoracotomy, and subsequent reconstruction was carried out by means of an oesophagogastrostomy using a gastric tube through the retrosternal route. To evaluate the characteristics of pretreatment tumour and resected oesophagus, we selected cases based on the following criteria: (1) Patients with two or more normal size pretreatment biopsy samples, or one large biopsy (2 mm^3 size). (2) Those with a resected oesophagus. (3) Those who received neoadjuvant cisplatin treatment of equal to or more than two cycles (two to five). (4) Patients for which there were adequate tumour tissues (more than 2000 nuclei). (5) Patients whose biopsies were histologically squamous cell carcinoma (the incidence of adenocarcinoma in Japan is less than 5%). Amongst the 91 patients, 8 cases had already received chemotherapy when the biopsy samples were obtained (primary biopsy was conducted by another hospital), 8 cases received only one cycle administration of cisplatin. In 5 cases, paraffin blocks could not be obtained, 7 cases had insufficient cells in the samples because they received only a single biopsy and 4 cases were not squamous cell carcinoma. Thus, 59 cases were assessable for response to neoadjuvant chemotherapy. Amongst those, 49 cases received a curative operation (R0: no residual tumour) without operative death and 10 cases received a non-curative operation (R1 or R2: microscopic or macroscopic residual tumour). In the curative resection cases, 17 patients (17/49, 35%) received postoperative irradiation (50.4 Gy, T-shaped field that included the bilateral lower neck and the upper mediastinum [10]) and 10 patients (20%) received postoperative chemotherapy.

2.2. Histological criteria

The histological effect of cisplatin was classified according to the following histological criteria established by 'The Japanese Society for Oesophageal Diseases': Histopathological criteria for the effects of radiation and anticancer chemotherapy. (Guidelines for the clinical and pathological studies on carcinoma of the oesophagus [11]);

Grade 0: No change.

Grade 1a: Necrosis or disappearance of the tumour is present in less than one-third of the whole lesion, or only cellular or structural changes are visible in variable amounts.

Grade 1b: Necrosis or disappearance of the tumour is present in no more than two-thirds of the whole lesion.

Grade 2: Necrosis or disappearance of the tumour is present in more than two-thirds of the whole lesion, but viable tumour cells are still remaining.

Grade 3: Marked change. The whole lesion falls into necrosis and/or is replaced by fibrosis, with or without granulomatous changes. No viable tumour cells are observed.

The histological effects were evaluated by 2 authors without prior knowledge of clinical and immunostaining data.

2.3. Antibodies and the immunohistochemical procedure

Formalin-fixed, paraffin-embedded pretreatment biopsy samples were used in the immunohistochemical staining which was performed by the avidin–biotin method as previously described [12]. The antibodies used in this study were as follows: p53 (DO-7, DAKO Kyoto, Japan), cyclin D1 (anti-cyclin D1/Bcl-1, MBL, Nagoya, Japan). The unmasking of antigens was carried out by incubation in citrate buffer (pH 6.0) at 100°C for 2 min of five cycles (microwave). Sections were incubated overnight at 4°C with each antibody in phosphate buffered saline (PBS) containing 5% horse serum and were counterstained with Mayer's haematoxylin. The intensity of immunohistochemical staining was evaluated in all areas of the slide section for correlation and

confirmation of the tissue analysis. More than 10% of positive staining in the nuclei was defined as a positive staining for p53 [13] and cyclin D1 [14]. The immunohistochemical staining was evaluated by 2 authors without prior knowledge of the histological response.

2.4. Scoring of the results

Positively stained cases were designated '1' and negatively stained or normal stained cases were designated '0'. Age, sex and TNM stage were divided into the following two categories: (Under 60 years old: 0, 60 years and older: 1), (female: 0, male: 1), (stage 1, 2a and 2b: 0, stage 3, 4a and 4b: 1). With regard to the histological effect, the term was divided into the following two categories: (grade 0: 0, grade 1a, 1b and 2: 1). Finally, the term of postoperative treatment was divided into the following two categories: (not done: 0, done: 1).

2.5. Statistical analysis

Survival curves of the patients were calculated by Kaplan–Meier method and analysed by generalised-Wilcoxon testing and log rank testing. Statistical analysis was carried out using the Kruskal–Wallis Chi-square and Fisher's exact tests. Cox's proportional hazard model was used for the multivariate analysis. The software used was JMP version 3 for Macintosh (SAS institute Inc. Cary NC, USA). A P value of <0.05 was taken as the level of significance.

3. Results

According to our selection criteria, 59 cases were selected out of 91 cases and 49 cases received curative operation (R0: no residual tumour) without operative death. Postoperative pathological TNM stage was used for stage analysis. 7 cases which had been diagnosed as node-negative preoperatively, were diagnosed as node-positive postoperatively. One patient who had obvious lymph node metastases preoperatively, had fibrosis only in the resected lymph nodes and pretreatment TNM stage was used for this case. In the other PR (partial response) or MR (minor response) cases, tumour cells were found to be the same as in pretreatment clinical staging. Most patients (83%, 49/59) received cisplatin 30 mg/m^2/week treatment of two cycles preoperatively, 3 NC cases received three cycles, 3 NC and 1 PR cases received four cycles, 2 MR and 1 PR cases received five cycles. Moderate response (PR + MR) of cisplatin treatment was 17% (10/59) (Table 1). With regard to postoperative adjuvant therapy, 10 cases received postoperative chemotherapy which was primarily carried out based on the histological effect: 6 patients received cisplatin 30 mg/m^2/two cycles, 2 patients received three cycles and 2 patients received cisplatin plus 5-FU combination chemotherapy for two cycles. Postoperative radiation therapy was performed when lymph node metastases were detected in a postoperative pathological examination in the upper mediastinum or cervical area.

First, we analysed the histological response of preoperative cisplatin treatment. There were 35 cases (59%) of grade 0, 15 cases (25%) of grade 1a, 8 cases (14%) of grade 1b and one case (2%) of grade 2. There was no grade 3 case (Table 1).

Secondly, the impact of the histological effect on the patient's prognosis was analysed using 49 curative oesophagectomy cases (R0). There was no significant difference in the clinicopathological background between cisplatin-sensitive and -resistant cases (Table 2). The prognosis of cisplatin-sensitive cases (grade 1a, 1b, 2) was significantly better than those of the cisplatin-resistant cases (grade 0) ($P=0.041$, Fig. 1).

Next, we analysed p53 and cyclin D1 status by means of immunohistochemical staining in preoperative biopsy samples. A representative staining of each antibody is shown in Fig. 2. p53 and cyclin D1 positive staining in the biopsy samples was observed in 44% (26/59) and

Table 1
Clinical and histological response of the patients

Term	($n=59$) n (%)
Sex	
Male	49 (83)
Female	10 (17)
Age (years)	
Mean (S.D.)	63.1 (8.8)
TNM stage	
1	5 (8)
2a	3 (5)
2b	14 (24)
3	24 (41)
4a	5 (8)
4b	8 (14)
Clinical response	
PD	3 (5)
NC	46 (78)
MR[a]	6 (10)
PR	4 (7)
CR	0
Histological response (grade)	
0	35 (59)
1a	15 (25)
1b	8 (14)
2	1 (2)
3	0

PD, progressive disease; NC, no change; MR, minor response; PR, partial response; CR, complete response.

[a] Tumour size decreased more than 25% but less than 50%. A P value of <0.05 was taken as level of significance.

Table 2
Clinicopathological data of the curative resection cases[a]

Term	Grade 0 ($n=27$) n (%)	Grade 1a ($n=14$) n (%)	Grade 1b, 2 ($n=8$) n (%)	P value
Sex				
Male	24 (89)	10 (71)	7 (88)	0.347
Female	3 (11)	4 (29)	1 (13)	
Age (years)				
Mean (S.D.)	62.7 (8.42)	61.6 (9.04)	67 (8.7)	0.451
TNM stage				
1	2 (7)	3 (21)	0	0.446
2a	2 (7)	0	1 (13)	
2b	7 (26)	4 (29)	1 (13)	
3	9 (33)	5 (36)	4 (50)	
4a	3 (11)	1 (7)	0	
4b	4 (15)	1 (7)	2 (25)	
T				
T1	7 (26)	3 (21)	0	0.483
T2	8 (30)	5 (36)	2 (25)	
T3	6 (22)	3 (21)	5 (63)	
T4	6 (22)	3 (21)	1 (13)	
N				
N0	4 (15)	5 (36)	2 (25)	0.317
N1	23 (85)	9 (64)	6 (75)	
M				
M0	20 (74)	12 (86)	7 (88)	0.597
M1a	3 (11)	1 (7)	0	
M1b	4 (15)	1 (7)	1 (13)	

[a] P calculated using the Kruskal–Wallis test. A P value of <0.05 was taken as level of significance.

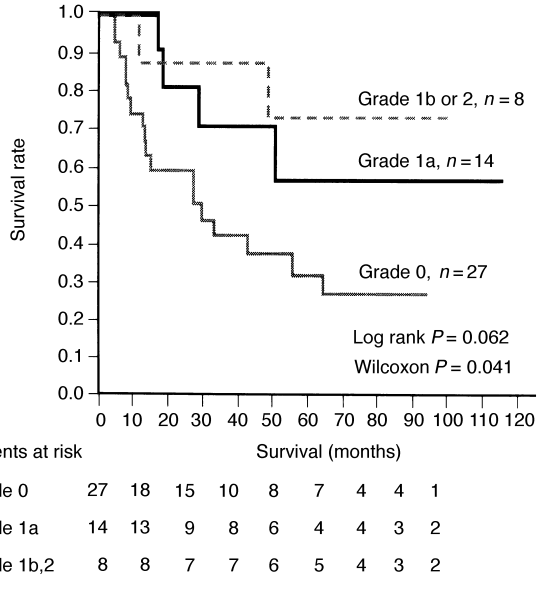

Fig. 1. Cumulative survival curves of the patients with oesophageal cancer calculated by the Kaplan–Meier method. Statistical analysis was done by log rank test and Generalised Wilcoxon test.

Table 3
Histological effects and protein accumulation

	Grade 0, 1a n (%)	Grade 1b, 2 n (%)	Total n (%)
p53 IHC(−)	25 (42)	8 (14)	33 (56)
p53 IHC(+)	25 (42)	1 (2)	26 (44)
	$P=0.032$		
Cyclin D1 IHC(−)	35 (59)	8 (14)	43 (73)
Cyclin D1 IHC(+)	15 (25)	1 (2)	16 (27)
	$P=0.230$		
p53 IHC(−) and Cyclin D1 IHC(−)	19 (32)	7 (12)	26 (44)
p53 IHC(+) and/or Cyclin D1 IHC(+)	31 (53)	2 (3)	33 (56)
	$P=0.032$		

IHC(−), negative staining in immunohistochemical analysis; IHC(+), positive staining in immunohistochemical analysis. A P value of <0.05 was taken as level of significance. (Fisher's exact test.)

27% (16/59), respectively. Pretreatment biopsy samples with p53 protein accumulation showed resistance to cisplatin ($P=0.032$). In contrast, cyclin D1 positive samples, in general, did not show resistance to cisplatin ($P=0.230$), Thus, a combined analysis of cyclin D1 and p53 expression could predict the histological effect ($P=0.032$) (Table 3).

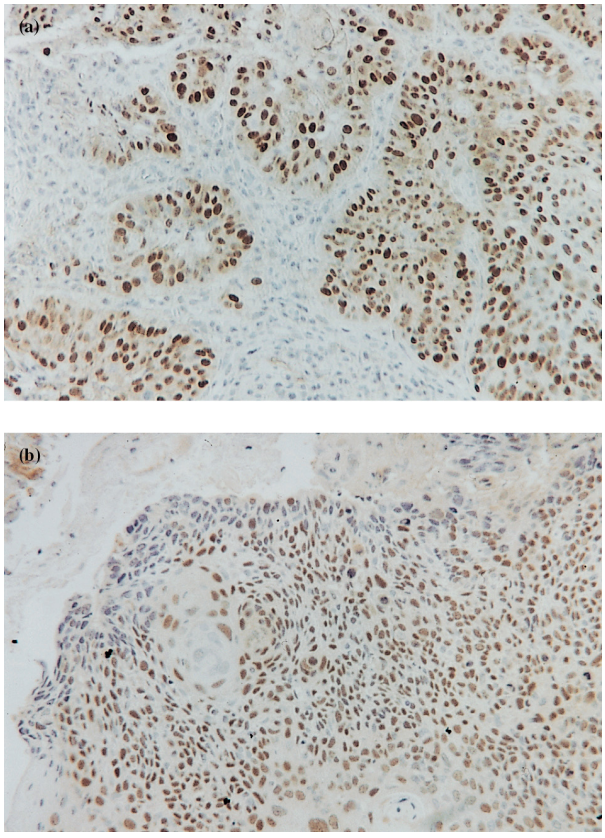

Fig. 2. Representative staining of each antibody used in this study. (a) p53; (b) cyclin D1. Intense nuclear staining can be seen in the tumour cells (original magnification ×100: a and b).

Table 4
Cox's multivariate analysis

Term	Estimate	Risk ratio (95% CI)	*P* value
Age (>59 years)	1.614	5.023 (1.70–17.81)	0.003
Sex (male)	2.49	12.069 (1.92–243.47)	0.005
TNM stage (3, 4a, 4b)	1.84	6.296 (2.12–22.28)	<0.001
p53	0.3	1.351 (0.52–3.49)	0.535
Cyclin D1	−0.286	0.751 (0.20–2.47)	0.646
Histological response (grade 1a, 1b, 2)	−2.322	0.098 (0.03–0.30)	<0.001
Postoperative chemotherapy	0.808	2.243 (0.74–6.27)	0.145
Postoperative radiation	−0.766	0.465 (0.15–1.35)	0.161

A *P* value of <0.05 was taken as level of significance.
CI, confidence interval.

Finally, we analysed whether the histological response to neoadjuvant cisplatin treatment was an independent prognostic factor in oesophageal SCC. There was some selection bias in the postoperative treatment, therefore, postoperative chemotherapy and radiotherapy were included in a multivariate analysis. Cox's multivariate analysis revealed that the histological response was an independent prognostic factor whilst p53 protein accumulation and cyclin D1 expression were not (Table 4).

4. Discussion

Cisplatin-based induction chemotherapy followed by surgical resection has been thought to improve the prognosis of patients with oesophageal cancer [2,3]. We have proposed low-dose cisplatin as a single agent neoadjuvant chemotherapy for advanced oesophageal cancer [4]. This protocol has a low response rate (17%, Table 1), but has been estimated to be a simple and safe pre-operative treatment. Even following low-dose administration of cisplatin (only one case achieved a TNM downstaging), our results showed that histological response was correlated with patient's survival. This means that histological response is one characteristic of a tumour behaviour. Additionally, low-dose cisplatin may influence the suppression of micrometastases of oesophageal cancer. Pathological response provides the most precise measure of the clinical effects of induction treatment [15]. As in several recent publications, those patients responding to chemotherapy (cisplatin-based regimens) had a better prognosis after surgery than non-responders who underwent resection [16,17]. If a chemotherapy response could be predicted by a pretreatment marker, those patients who would respond would certainly be candidates for pre-operative therapy. However, the use of clinical parameters cannot accurately predict which patients may be best served by pre-operative chemotherapy.

The *TP53* gene is one of the most frequent genetic alterations (approximately 50% of tumours) in oesophageal SCC [18]. Recently, *in vitro* studies conducted in fibroblast cell lines, showed that p53-dependent apoptosis mechanisms appear to be involved in the cytotoxity induced by ionising radiation and by several anticancer agents [19]. Furthermore, adenovirus-mediated transfer of the wild-type *TP53* gene into a cultured tumour of human lung cancer cell line with a homozygous deletion of *TP53* markedly increased the sensitivity of the cells to cisplatin [20]. This suggested a direct link between wild-type p53 expression and cisplatin-mediated cytotoxicity in cancer cells. There are also several reports regarding cisplatin sensitivity and *TP53* mutation [15,21,22]. Our results suggest that this speculation is also applicable to oesophageal cancer.

For the purpose of clinical use, we used immunohistochemical analysis to evaluate the p53 status in pretreatment biopsy samples, however, there are some criticisms regarding this detection method. Immunohistochemical staining does not always reflect genetic abnormality; only missense mutations resulted in a positive immunostaining [20]. Although we analysed more than two biopsy samples or one large biopsy in each case, biopsy samples do not always reflect the characteristics of main tumours because of the heterogeneity of genetic expression [23]. With regard to the relationship between p53 protein accumulation and *TP53* mutation, there was concordance between *TP53* mutation and protein expression (69% [24]–76.2% [25]). In contrast, Mineta and colleagues [26] suggested that *TP53* mutation, but not p53 overexpression, correlates with survival in head and neck squamous cell carcinoma. However, Abdel-Fattah and associates [27] suggested that high frequency of p53 protein accumulation in the absence of detected mutations is associated with poor prognosis in microdissected transitional cell carcinoma of the human urinary bladder. For oesophageal cancer, Ribeiro and coworkers [5] suggested that *TP53* sequence analysis is a better determination of *TP53* mutational damage than immunohistochemistry alone and can be used as a prognostic marker, however, the majority of cases were adenocarcinoma.

Furthermore, recent research has suggested that specific *TP53* genetic suppressor elements confer resistance to cisplatin in ovarian cancer [28] and specific *TP53* gene mutations may confer different levels of chemoresistance to doxorubicin in breast cancer [29]. Point mutations in *TP53* predominantly occur in that segment of the genome responsible for DNA binding, coded, for the most part, by exons 5–8 [30]. In non-small cell lung cancer, Vega and colleagues [31] suggested mutation in exon 5 of *TP53* was a prognostic indicator of shortened survival whereas Huang and coworkers [32] proposed that exons 7 and 8 are poor prognostic factors. If future studies confirm that specific *TP53* mutations are associated

with poor outcome when a particular cytotoxic agent is used, then identifying patients with tumours that carry these mutations may improve the treatment selection.

The occurrence of protein accumulation without detectable mutation suggests an altered regulation of expression or the presence of p53-interacting proteins that may contribute to p53 stabilisation [21]. In p53-negative tumours, other genetic changes or differences in the tumour microenvironment may prevent p53-mediated cell death. For example, MDM2, HPV infection, p19ARF, ATM, p21, Bax and Bcl2 can all affect p53 function [33]. In general, it is thought that aberrant p53 protein expression as assessed by immunohistochemical techniques provides a clinically useful measure of deregulated p53 function in oesophageal SCC. However, whether other cell cycle regulators act in concert with or independent of p53 to elicit these beneficial clinical responses remains to be determined. Even if sequence analyses become easier to perform, microdissection followed by this technique is not easily applicable in the clinical field. Although immunohistochemical analysis has some disadvantages, this method is able to detect the protein expression of several genes simultaneously and to detect distributions of protein accumulation.

With regard to response criteria, Righetti and coworkers [21] suggested that a partial response reflected the presence of a subpopulation of resistant cells, only patients with a complete response were considered 'responsive' for purposes of correlation with p53. Our results also indicated that if we include the grade 1b and 2 (mild response) cases with the responder, there was a strong correlation between histological response and p53 protein accumulation. Interestingly, from the viewpoint of patients' survival, grade 1a cases also demonstrated better survival compared with grade 0 cases. For this reason, p53 protein accumulation was not a prognostic factor in our low-dose cisplatin administration regimen.

Another modulation of chemosensitivity through altered expression of cell cycle regulatory genes in cancer is the cyclin D1–pRb–p16 pathway [34]. There have already been several reports regarding cyclin D1 in oesophageal carcinoma [7,35] and an amplification and overexpression of the cyclin D1 gene were reported as a significant prognostic factor. Hochhauser [34] indicated that cyclin D1 expression related to methotrexate sensitivity.

Recently, Sarbia and colleagues [8] reported that expression of cyclin D1 in 38 oesophageal carcinomas with multimodal treatment (FLEP: folic acid, etoposide, 5-FU and cisplatin) was correlated with a poor response to chemotherapy, but not with overall survival. Our results also suggested that cyclin D1 expression may be related to cisplatin sensitivity when combined with p53 status, however, further study is needed to confirm this.

In conclusion, cisplatin sensitivity of patients is an independent prognostic factor of oesophageal cancer and p53 expression may be a marker of cisplatin sensitivity. However, a note of caution should be added. The small numbers in this study limit any definitive conclusions. This may also be reflected in the differences in significance observed between p53 and cyclin D1 expression and the study of larger groups of cases or multi-institutional analyses will be needed to confirm these data. Nevertheless, analysis of the immunophenotype, irrespective of the genotype, may represent a clinically useful approach to predict response to cisplatin-based therapy. For the treatment of a non-responder, more aggressive combination protocol (chemoradiation) or new drugs should be considered.

Acknowledgements

The authors thank Professor Hirohiko Yamabe, Kyoto University, for kindly preparing the paraffin section. The authors would also like to thank Ms Sandra Snider for her support in proofreading the manuscript and Shunzou Maetani, M.D, Ph.D, Vice president of Research Center of Tenri Hospital, for his excellent advice in statistical analysis. This work was supported in part by a Grant-in-Aid from the Japanese Ministry of Education, Science and Culture (Grant 09671301 and 09470265).

References

1. Kelsen DP, Ginsberg R, Pajak TF, *et al*. Chemotherapy followed by surgery compared with surgery alone for localized esophageal cancer. *N Engl J Med* 1998, **339**, 1979–1984.
2. Ancona E, Ruol A, Castoro C, *et al*. First-line chemotherapy improves the resection rate and long-term survival of locally advanced (T4, any N, M0) squamous cell carcinoma of the thoracic esophagus. *Ann Surg* 1997, **226**, 714–724.
3. Law S, Fok M, Chow S, Chu KM, Wong J. Preoperative chemotherapy versus surgical therapy alone for squamous cell carcinoma of the esophagus: a prospective randomized trial. *J Thorac Cardiovasc Surg* 1997, **114**, 210–217.
4. Imamura M, Shimada Y, Kanda Y, *et al*. Usefulness of preoperative low dose cisplatin treatment for advanced oesophageal cancer. *Jpn J Surg* 1992, **22**, 409–415.
5. Ribeiro U, Finkelstein SD, Safatle-Ribeiro AV, *et al*. p53 sequence analysis predicts treatment response and outcome of patients with oesophageal carcinoma. *Cancer* 1998, **83**, 7–18.
6. Muro K, Ohtsu A, Boku N, *et al*. Association of p53 protein expression with responses and survival of patients with locally advanced oesophageal carcinoma treated with chemoradiotherapy. *Jpn J Clin Oncol* 1996, **26**, 65–69.
7. Shinozaki H, Ozawa S, Ando N, *et al*. Cyclin D1 amplification as a new predictive classification for squamous cell carcinoma of the esophagus adding gene information. *Clin Cancer Res* 1996, **2**, 1155–1161.
8. Sarbia M, Stahl M, Fink U, *et al*. Prognostic significance of cyclin D1 in esophageal squamous cell carcinoma patients treated

with surgery alone or combined therapy modalities. *Int J Cancer* 1999, **84**, 86–91.
9. Imamura M, Shimada Y, Kanda Y, *et al*. Hemodynamic changes after resection of thoracic duct for en bloc resection of oesophageal cancer. *Jpn J Surg* 1992, **22**, 226–232.
10. Nishimura Y, Ono K, Imamura M, *et al*. Postoperative radiation therapy for oesophageal cancer. *Radiat Med* 1989, **7**, 88–94.
11. *Histopathologic Criteria for the Effects of Radiation and Anticancer Chemotherapy*. *Guidelines for the Clinical and Pathologic Studies on Carcinoma of the Esophagus*, 8th edn., 1992. Tokyo, Japan. Japanese society for oesophageal diseases, Kanehara & Co.
12. Uchida S, Shimada Y, Watanabe G, *et al*. In oesophageal squamous carcinoma vascular endotherial growth factor is associated with p53 mutation, advanced stage and poor prognosis. *Br J Cancer* 1998, **77**, 1704–1709.
13. Casey G, Lopez ME, Ramos JC, *et al*. DNA sequence analysis of exon 2 through 11 and immunohistochemical staining are required to detect all known p53 alterations in human malignancies. *Oncogene* 1996, **13**, 1971–1981.
14. Takeuchi H, Ozawa S, Ando N, *et al*. Altered p16/MTS1/CDKN2 and cyclinD1/PRAD1 gene expression is associated with the prognosis of squamous cell carcinoma of the esophagus. *Clin Cancer Res* 1997, **3**, 2229–2236.
15. Rusch V, Klimstra D, Venkatraman E, *et al*. Aberrant p53 expression predicts clinical resistance to cisplatin based chemotherapy in locally advanced non-small cell lung cancer. *Cancer Res* 1995, **55**, 5038–5042.
16. Roth JA, Pass HU, Flanagan MM, Graeber GM, Rosenberg JC, Steinberg S. Randomized clinical trial of preoperative and postoperative adjuvant chemotherapy with cisplatin, vindesine, and bleomycin for carcinoma of the esophagus. *J Thorac Cardiovasc Surg* 1988, **36**, 242–248.
17. Kelsen DP, Minsky B, Smith M, *et al*. Preoperative therapy for oesophageal cancer: a randomized comparison of chemotherapy versus radiation therapy. *J Clin Oncol* 1990, **8**, 1352–1361.
18. Wagata T, Shibagaki I, Imamura M, *et al*. Loss of 17p, mutation of p53 gene, and overexpression of p53 protein in oesophageal squamous cell carcinomas. *Cancer Res* 1993, **53**, 846–850.
19. Lowe SW, Ruley HE, Jacks T, Housman DE. p53-dependent apoptosis modulated the cytotoxity of anticancer agent. *Cell* 1993, **74**, 957–967.
20. Fujiwara T, Grimm EA, Mukhopadhyay T, Zhang WW, Owen-Schaub LB, Roth JA. Induction of chemosensitivity in human lung cancer cells *in vivo* by adenovirus-mediated transfer of the wild-type p53 gene. *Cancer Res* 1994, **54**, 2287–2291.
21. Righetti SC, Torre GD, Pilotti S, *et al*. A comparative study of p53 gene mutations, protein accumulation, and response to cisplatin-based chemotherapy in advanced ovarian carcinoma. *Cancer Res* 1996, **56**, 689–693.
22. Cascinu S, Graziano F, Del Ferro E, *et al*. Expression of p53 protein and resistance to preoperative chemotherapy in locally advanced gastric carcinoma. *Cancer* 1998, **83**, 1917–1922.
23. Hori H, Miyake S, Akiyama Y, Endo M, Yuasa Y. Clonal heterogeneity in human oesophageal squamous cell carcinomas on DNA analysis. *Jpn J Cancer Res* 1996, **87**, 923–929.
24. Sauter ER, Ridge JA, Litwin S, Langer CJ. Pretreatment p53 protein expression correlates with decreased survival in patients with end-stage head and neck cancer. *Clin Cancer Res* 1995, **1**, 1407–1412.
25. Shimada Y, Imamura M, Tanaka H, *et al*. Relationship of cisplatin resistance to p53 mutations in oesophageal cancer patients. XXX World Congress of the International College of Surgeons. Abe O, Inokuchi K, Takasaki K Edi. Monduzzi Editor 1996, 293–298.
26. Mineta H, Borg A, Dictor M, Wahlberg P, Akervall J, Wennerberg J. p53 mutation, but not p53 overexpression, correlates with survival in head and neck squamous cell carcinoma. *Br J Cancer* 1998, **78**, 1084–1090.
27. Abdel-Fattah R, Challen C, Griffiths TR, Robinson MC, Neal DE, Lunec J. Alterations of TP53 in microdissected transitional cell carcinoma of the human urinary bladder: high frequency of TP53 accumulation in the absence of detected mutations is associated with poor prognosis. *Br J Cancer* 1998, **77**, 2230–2238.
28. Gallagher WM, Cairney M, Schott B, Roninson IB, Brown R. Identification of p53 genetic supressor elements which confer resistance to cisplatin. *Oncogene* 1997, **14**, 185–193.
29. Aas T, Borresen AL, Geisler S, *et al*. Specific p53 mutations are associated with de novo resistance to doxorubicin in breast cancer patients. *Nature Med* 1996, **2**, 811–814.
30. Hollstein MC, Sidranski D, Vogelstein B, Harris CC. P53 mutation in human cancers. *Science* 1991, **253**, 49–53.
31. Vega FJ, Iniesta P, Caldes T, *et al*. p53 exon 5 mutations as a prognostic indicator of shortened survival in non-small-cell lung cancer. *Br J Cancer* 1997, **76**, 44–51.
32. Huang CL, Takai T, Adachi M, Konishi T, Higashiyama M, Miyake M. Mutations in exon 7 and 8 of p53 as poor prognostic factors in patients with non-small cell lung cancer. *Oncogene* 1998, **16**, 2469–2477.
33. Kirsch DG, Kastan MB. Tumour-suppressor p53: implications for tumour development and prognosis. *J Clin Oncol* 1998, **16**, 3158–3168.
34. Hochhauser D. Modulation of chemosensitivity through altered expression of cell cycle regulatory genes in cancer. *Anti-Cancer Drugs* 1997, **8**, 903–910.
35. Naitoh H, Shibata J, Kawaguchi A, Kodama M, Hattori T. Overexpression and localization of CyclinD1 mRNA and antigen in oesophageal cancer. *Am J Pathol* 1995, **146**, 1161–1169.