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THE PROGNOSTIC SIGNIFICANCE OF P53 OVEREXPRESSION IN METASTATIC LYMPH NODES OF ESOPHAGEAL CARCINOMAS AND ASSOCIATION WITH CELL PROLIFERATION AND APOPTOSIS

JUN YAMASHITA
First Department of Surgery, Nara Medical University
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Abstract: The p53 overexpression is not always correlated with the clinicopathologic features or clinical outcome of esophageal cancer patients. The incidence of lymph node metastasis is the most significant prognostic factor, but the molecular genetic events including p53 overexpression in metastatic lymph node have remained unclear. The aim of this study was to investigate the prognostic significance of p53 overexpression in lymph node metastatic tumors in comparison with primary tumors and the relationship between p53 overexpression and proliferative activity or apoptosis. We performed immunohistochemical analysis for both p53 protein and proliferating cell nuclear antigen (PCNA) and in situ DNA nick end labeling method to detect apoptosis in 35 primary tumors and 25 metastatic lymph nodes. The p53 overexpression in lymph node metastatic tumors correlated with shorter survival after operation in lymph node-positive cancer patients ($p<0.01$). In primary tumors, there was no statistically significant difference for PCNA labeling index (LI) and apoptotic LI between p53-positive and negative tumors. In lymph node metastatic tumors, the PCNA LI of p53-positive tumors was significantly higher than that of p53-negative tumors ($p<0.04$), and the apoptotic LI of p53-positive tumors tended to be lower than that of p53-negative tumors. The p53 overexpression is related to tumor cell proliferation and inhibition of apoptosis in lymph node metastatic tumors, and p53 overexpression in metastatic lymph nodes may correlate with the malignant potential of esophageal carcinoma. (奈医誌. J. Nara Med. Ass. **51**, 1～13, 2000)

Key words: esophageal cancer, p53, lymph node metastasis, PCNA, apoptosis

INTRODUCTION

Esophageal squamous cell carcinomas are known to have high malignant potential and a poor clinical outcome. Recent studies have revealed that the genesis and/or progression of esophageal carcinoma may be due to the accumulation of several genetic alterations, including inactivation of tumor suppressor genes such as p53 or the MTS1 gene and/or activation of protooncogenes such as cyclin D gene.[1-3] In particular, the p53 gene may be the most frequent target of genetic changes in cancers, and a high percentage of esophageal carcinomas have been found to show mutations and overexpression of p53 protein.[1,4,5] Overexpression of the p53

protein is considered to play an important part in the activation of cell proliferation and carcinogenesis of various tumors including esophageal carcinoma.[4-7] It is accepted that wild -type p53 protein may play a central role in controling cell proliferation by contributing to a regulated G1-S checkpoint to ensure the order of cellular events before DNA synthesis, and lead to the initiation of apoptosis.[8-11] In contrast, the mutated p53 protein is considered to alter this function resulting in activation of cell proliferation in tumor progression,[12,13] and activated cell proliferation may affect the clinical outcome of esophageal carcinoma. However, the overexpression of p53 protein is not always correlated with the clinicopathologic features or clinical outcome of esophageal cancer patients. Therefore, the detailed effects of mutated p53 protein in the tumor progression of esophageal carcinoma have remained uncertain.

On the other hand, it is well known that the incidence of lymph node metastasis is the most significant prognostic factor in esophageal carcinoma. However, the molecular genetic events in metastatic lymph node contributing to the malignant development of esophageal carcinoma have remained obscure; the somatic mutations found thus far have been limited to primary tumors.

In the present study, we attempted to clarify the prognostic significance of p53 overexpression in lymph node metastatic tumors of esophageal carcinoma in comparison with primary tumors, and to analyze the relationship between p53 overexpression and proliferative activity or apoptosis in primary and lymph node metastatic tumors, using immunohistochemistry for both the p53 protein and proliferating cell nuclear antigen (PCNA),[14-17] and the in situ DNA nick end labeling method[18] for apoptosis.

MATERIALS AND METHODS

Cases

A total of 60 tumor specimens, comprising 35 primary esophageal tumors and 25 lymph node metastatic tumors, were obtained from 35 patients (10 patients did not have lymph node metastasis and 25 had lymph node metastasis) with esophageal squamous cell carcinoma, who underwent esophagectomy at Nara Medical University Hospital in Japan from January, 1991 to December, 1993. These patients had not received irradiation or chemotherapy before surgery, and the patients were those for whom absolute noncurative resection had been excluded in this study. The presence of lymph node metastasis was judged histologically after surgery by studying all lymph nodes obtained. The pathologic classification was based on the Guidelines for Clinical and Pathologic Studies on Carcinoma of the Esophagus established by the Japanese Society for Esophageal Diseases.[19] The tumor specimens were routinely fixed with 10% buffered formalin and embedded in paraffin. The serial sections of each tumor were cut to a thickness of 4μm, stained immunohistochemically for p53 and PCNA, and the in situ DNA nick end labeling method was carried out to detect apoptosis.

Immunohistochemical Staining Procedures

A combination of the ordinary avidin-biotin-peroxidase complex (ABC) method and microwave oven heating was performed.[20] After routine deparaffinization and blocking of endogenous peroxidase with 0.3% hydrogen peroxide in methanol for 30 minutes at room temperature, the sections were immersed in distilled water, placed in a microwave oven

(SHARP-RE-T67, Osaka, Japan) and microwaved for antigen retrieval for 5 minutes at 500 Watts. The sections were incubated with 10% normal rabbit serum for 30 minutes to block nonspecific reactions in the tissues. The primary antibodies used were mouse anti-human p53 monoclonal antibody (DO7: Novocastra Laboratories, Newcastle, UK, ×50 dilution) for mutant and wild-type p53 protein and mouse anti-PCNA monoclonal antibody (PC10: Novocastra Laboratories, ×100 dilution) for PCNA. The sections were incubated with the primary antibody for 60 minutes at room temperature. They were well washed with PBS, incubated with biotinylated anti-mouse immunoglobulin G containing 1.0% normal rabbit serum for 10 minutes, and incubated with ABC reagent for 5 minutes. They underwent peroxidase staining for 5 minutes using a solution of 40 mg of 3,3'-diaminobenzidine tetrahydrochloride in 200 ml of 0.05 mol/l tris HCI buffer (pH 7.6) containing 40μl of 30% hydrogen peroxide. They were counterstained with Meyer's hematoxylin for 10 seconds, then examined with a light microscope.

Percentages of p53-positive cells were evaluated after counting 1,000 nuclei in randomly selected high-power fields (using ×40 objective and ×10 ocular lens). Cases were defined as positive (overexpression) for p53 immunostaining when over 30% of the cells were stained in each section. PCNA labeling indices (PCNA LI) were calculated after counting PCNA-positive cells per 1,000 nuclei in the areas for which p53 immunoreactivity were examined using serial sections.

In situ DNA nick end labeling

For the detection of apoptotic cells in sections of formalin-fixed and paraffin-embedded tissue, the in situ DNA nick end labeling method (Apop Tag Plus-Peroxidase in situ apoptosis detection kit, Oncor, USA) was used. After routine deparaffinization, nuclei of the sections were stripped by incubation with 20μg/ml proteinase K (Sigma, USA) for 15 minutes at room temperature. Endogenous peroxidase was inactivated by covering the sections with 3% hydrogen peroxide in PBS for 5 minutes. After adding the equilibration buffer for 10 minutes, TdT enzyme was pipetted onto the sections, which were then incubated in a moist chamber for 60 minutes at 37℃. The reaction was terminated by transferring the sections to stop/wash buffer for 30 minutes at room temperature. The sections were incubated with anti-digoxigenin-antibody-peroxidase to detect digoxigenin-11-dUTP labeling for 30 minutes at room temperature. Color development was performed using 3,3'-diaminobenzidine containing hydrogen peroxide solution. Counterstaining was achieved with Meyer's hematoxylin, and the sections were examined with a light microscope. The mammary glands or intestinal crypt cells from rats were used as positive controls for this analysis. Substitution of TdT with distilled water was used as a negative control.

Apoptotic labeling indices (apoptotic LI) were determined by counting apoptotic cells per 1,000 nuclei in the areas for which p53 immunoreactivity was examined using serial sections. Positively staining tumor cells with the morphological characteristics of apoptosis were identified using standard criteria, including chromatin condensation, nucleolar disintegration, and formation of crescentic caps of condensed chromatin at the nuclear periphery.[21,22] Necrotic areas were excluded from this evaluation, and positively staining cells with ballooning of the cytoplasm or condensed nuclei accompanied by inflammation were not counted.

Statistical Analysis

Quantitative data were analyzed by the chi-square test or Student's t test. Patient prognosis was examined on December 1, 1998. Overall survival rates were calculated by the Kaplan-Meier method[23] and were compared with the generalized Wilcoxon test. Independent effects of each prognostic factor were determined by the Cox life-table regression model (proportional hazards general linear model).[24] Significance was set at the 5% level.

RESULTS

Expression of p53

The immunohistochemical staining for p53 in primary esophageal tumors was strictly limited to the nuclei of cancer cells with variable intensities, and almost all primary tumors demonstrated a heterogeneous staining pattern (Fig. 1A). There were no cells positive for p53 in adjacent normal esophageal epithelia. Overexpression of p53 protein (over 30% positivity) was detected in 33 of the total number of tumors (55%), including 17 primary esophageal tumors (48.6%) and 16 lymph node metastatic tumors (64%) (Table 1A). Eleven out of 25 lymph node -positive cases showed p53 overxpression in both primary tumor and metastatic lymph nodes. In 5 cases, lymph node metastatic tumors showed p53 protein positivity, whereas primary tumors were negative for p53. In only one case, primary tumor showed p53 positivity, but lymph node metastatic tumors were negative (Table 1B). The cancer nests of p53-positive lymph node metastatic tumors were stained diffusely, and showed the same intensity as the stronger-staining areas of primary tumors (Fig. 2A).

Relationship between p53 Expression and Pathologic Features

The relationship between the clinicopathologic findings and p53 staining in primary tumors is summarized in Table 2. There was no significant correlation between p53 expression and histological type, depth of invasion, lymph node metastasis, lymphatic invasion, blood vessel invasion and histological staging. However, in lymph node-positive cases, the incidence of p53 overexpression in cases with distant lymph node metastasis (n3, n4) was significantly higher than that in cases without distant lymph node metastasis ($p<0.03$).

p53 Expression and Prognosis

The cumulative survival curves of 25 lymph node-positive cases are shown in Fig. 3. Survival curves revealed patients with p53 expression in primary tumor had worse prognoses than did those without p53 expression, but the difference did not reach statistical significance. In contrast, there was a significant difference between patients with p53 expression in lymph node metastatic tumors and those without p53 expression ($p<0.01$). Putative prognostic factors, including p53 expression in lymph node metastatic tumors, were analyzed using the Cox regression model. The expression of p53 in lymph node metastatic tumors was found to be a significant prognostic factor independent of lymph nodal status or depth of cancer invasion (Table 3).

Staining patterns of PCNA-positive cells and apoptotic cells

The immunohistochemical staining for PCNA was almost entirely limited to the nuclei of

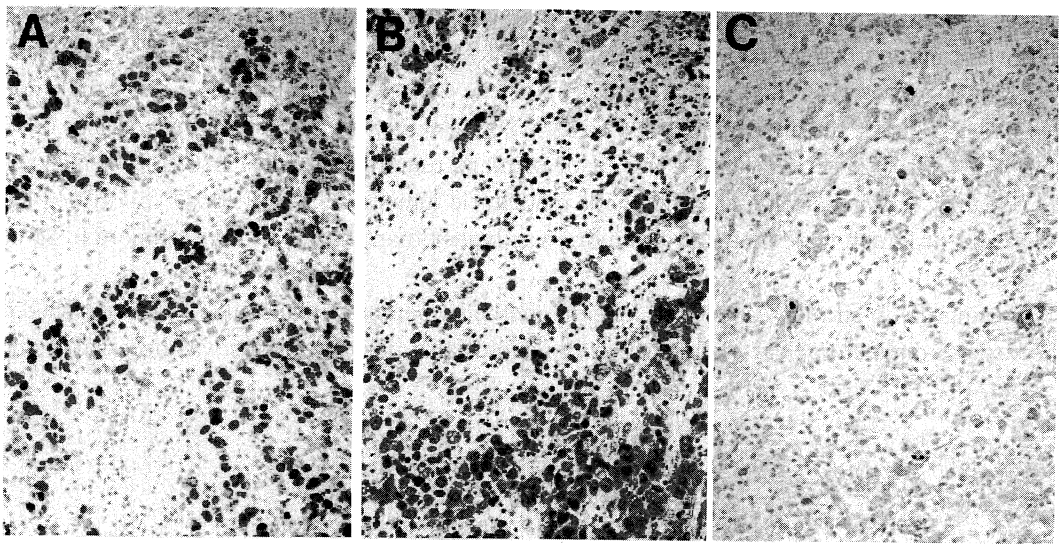

Fig. 1. Semi-serial sections of primary tumor of esophageal squamous cell carcinoma. A) Immunohistochemical staining for p53 protein was strictly limited to the nuclei of cancer cells with variable intensities, and demonstrated a heterogeneous staining pattern in primary cancer nests. ×100. B) Immunohistochemical staining for PCNA. The PCNA-positive cancer cells were spread homogeneously through the p53-positive areas. ×100. C) Apoptotic cancer cells showing distinct nuclear staining by the *in situ* DNA nick end labeling method were sporadically found in primary cancer nest. ×100.

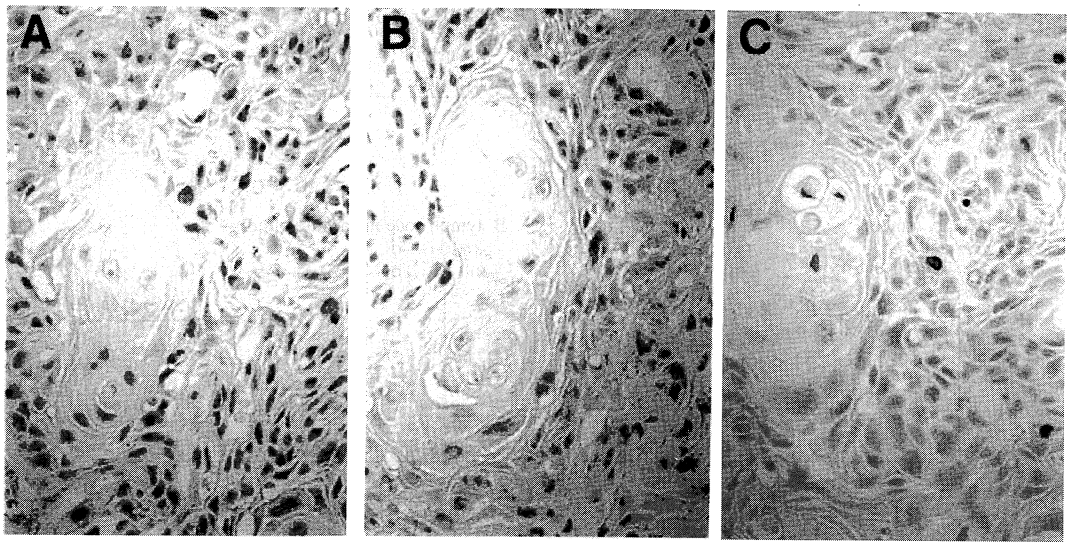

Fig. 2. Semi-serial sections of lymph node metastatic tumor of esophageal squamous cell carcinoma. A) Cancer cells demonstrating p53 positivity were diffusely distributed in metastatic cancer nest. ×200. B) The distributions of PCNA-positive cells were almost similar to those of p53-positive cells. ×200. C) Apoptotic cancer cells were sporadically found in metastatic cancer nest. Positively staining cells in cancer pearls were not counted in this study. ×200.

cancer cells with the diffuse or granular staining pattern. In primary tumors, PCNA-positive cells were spread homogeneously through the p53-positive areas, while PCNA-positive cells were most frequently found at the periphery of the cancer nests (Fig. 1B). In lymph node metastatic tumors, the distributions of PCNA-positive cells were almost similar to those of p53 -positive cells (Fig. 2B). The PCNA LI of primary tumors was 55.67±16.32%, which was significantly lower than that of lymph node metastatic tumors (79.38±9.81%) ($p<0.01$). The PCNA LI of lymph node metastatic tumors was higher than that of primary tumors in 24 out of 25 cases.

By using the in situ DNA nick end labeling method, apoptotic cells could be distinguished on the basis of distinct nuclear staining. Care to avoid false-positive results was necessary, since

Table 1. Results of immunohistochemical staining for p53 protein

A. p53 expression in primary and lymph node metastatic tumors

	No. of cases	P53 staining		P value
		positive	negative	
primary tumors	35	17(48.6%)	18(51.4%)	
lymph node metastatic tumors	25	16(64%)	9(36%)	NS

B. Distribution patterns of p53 staining between primary and lymph node metastatic tumors

	primary tumors	lymph node metastatic tumors	No. of cases
p53 staining	positive	positive	11(44%)
	positive	negative	1(4%)
	negative	positive	5(20%)
	negative	negative	8(32%)

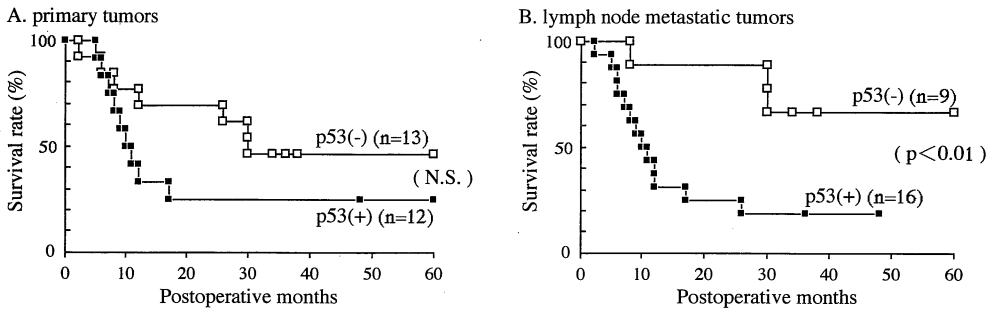

Fig. 3. The cumulative survival curves of patients with lymph node-positive esophageal carcinoma in relation to expression of p53. A) Patients with p53 overexpression in primary tumor had worse prognoses than those without p53 overexpression, but the difference did not reach statistical significance. B) Patients with p53 overexpression in metastatic lymph nodes had significantly worse prognoses than those without p53 overexpression ($p<0.01$).

some degenerating cells and a small number of necrotic cells were also labeled by this method.[21,22,25,26] In primary esophageal tumors and lymph node metastatic tumors, the apoptotic cells were evident as single cells apparently separated from neighboring cancer cells

Table 2. Correlation between p53 staining and clinicopathologic findings

Variable	No. of cases	P53 Overexpression		P value
		positive	negative	
Histological type				
well	12	4(33%)	8(67%)	
moderately	19	11(58%)	8(42%)	
poorly	4	2(50%)	2(50%)	NS
Depth of invasion				
ep, mm	2	0(0%)	2(100%)	
sm	3	1(33%)	2(67%)	
mp	9	7(78%)	2(22%)	
al	13	5(38%)	8(62%)	
a2, a3	8	4(50%)	4(50%)	NS
Lymph node metastasis				
n0	10	5(50%)	5(50%)	
n1, 2	14	4(29%)	10(71%)	a) P<0.03
n3, 4	11	8(73%)	3(27%)	
Lymphatic invasion				
negative	5	2(40%)	6(60%)	
positive	30	15(50%)	15(50%)	NS
blood vessel invasion				
negative	18	10(56%)	8(44%)	
positive	17	7(41%)	10(59%)	NS
Histological stage				
stage0	4	1(25%)	3(75%)	
stage1, 2	8	5(63%)	3(37%)	
stage3, 4	23	11(48%)	12(52%)	NS
total	35	17(48.6%)	18(51.4%)	

a) In lymph node-positive cases, the incidence of p53 overexpression in cases with distant metastasis (n3, n4) was significantly higher than that in cases without distant metastass ($p<0.03$).

Table 3. Proportional hazards geseral linear models comparing overall survival times with putative prognostic factors in lymph node-positive esophageal cancer patients

Variable	Beta	Standard error	Chi-square	P value
p53 expression in lymph node metastatic tumors (positive vs. negative)	1.37	0.68	4.06	0.04
Lymph node metastasis (n1, 2 vs. n3, 4)	−0.53	0.53	1.00	0.31
Depth of invasion (a0 vs. a1, 2, 3)	−0.43	0.67	0.42	0.51

(Figs. 1C and 2C). The apoptotic LI of primary tumors was 0.65±0.59%, which was significantly higher than that of lymph node metastatic tumors (0.31±0.22%) ($p<0.01$).

Relationship between p53 Expression and cell kinetics

Data on the relation between p53 expression and PCNA LI are summarized in Table 4. In primary tumors, the PCNA LI of p53-positive tumors was 60.8±14.4%, which was higher than that of p53-negative tumors (50.2±16.8%), but this difference was not statistically significant ($0.05<p<0.1$). In lymph node metastatic tumors, the value of PCNA LI for p53-positive tumors of 82.5±10.3% versus 73.8±5.8% for p53-negative tumors demonstrated a statistically significant difference ($p<0.04$).

Data on the relation between p53 expression and apoptotic LI are also shown in Table 4. In primary tumors, there was no statistically significant difference for apoptotic LI between p53 -positive and negative tumors. In lymph node metastatic tumors, the apoptotic LI of p53-positive tumors was 0.25±0.18%, which was lower than that of p53-negative tumors (0.42±0.25%), but this difference was not statistically significant ($0.05<p<0.1$).

The relationship between p53 and cell kinetics in lymph node metastatic tumors is shown in Fig. 4. The p53-positive metastatic lymph nodes tended to be distributed in the right lower field; this distribution pattern indicated that the lymph node metastatic tumors that were positive for p53 expression had higher proliferative activity and fewer cells undergoing apoptosis. In addition, there was a significant inverse correlation between apoptotic LIs and PCNA LIs ($r=-0.586$, $p<0.005$).

DISCUSSION

The p53 mutations occur frequently in various tumors, including esophageal carcinoma, and may contribute to important carcinogenic mechanisms.[1,4-7] The wild-type p53 protein is considered to play a central role in controlling cell proliferation by contributing to a metabolically regulated G1-S checkpoint to ensure the order of cellular events before DNA synthesis, activate the expression of adjacent genes that inhibit growth and/or invasion, and lead to the initiation of apoptosis.[8-11,27] In contrast, the mutated p53 protein is reported to alter this function and activate cell proliferation in the tumor progression.[12,13] However, the detailed

Table 4. Relationship between p53 expression and proliferative activity or apoptosis

	primary tumors		lymph node metastatic tumors	
	p53(+)(n=17)	p53(−)(n=18)	p53(+)(n=16)	p53(−)(n=9)
PCNA LI(%)	60.8±14.4[a)]	50.2±16.8[a)]	82.5±10.3[b)]	73.8±5.8[b)]
apoptotic LI(%)	0.72±0.58[c)]	0.58±0.61[c)]	0.25±0.18[d)]	0.42±0.25[d)]

a-d) Comparison between each labeled pair : a ; not significant ($p=0.054$), b ; $p<0.04$, c ; not significant, d ; not significant ($p=0.06$).

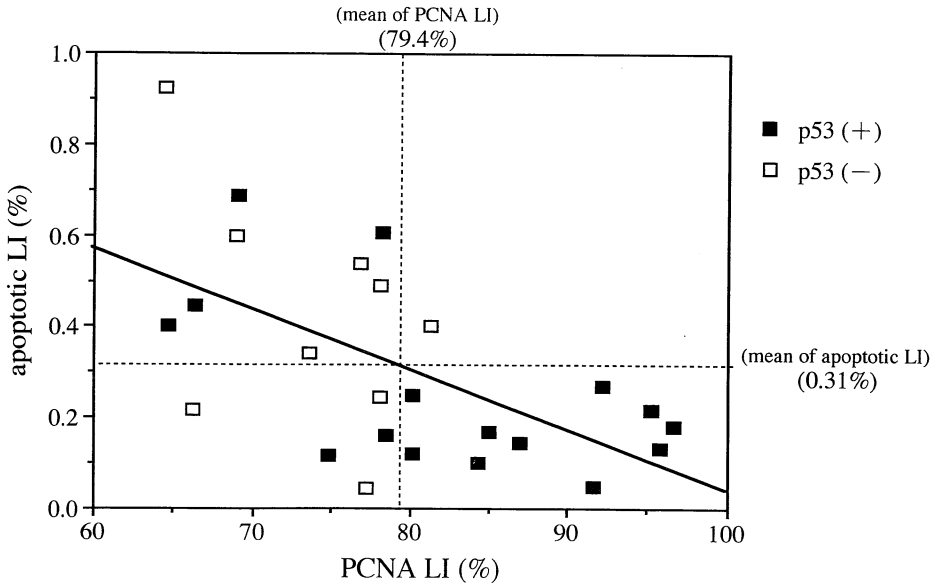

Fig. 4. Relationship between p53 expression and cell kinetics in lymph node metastatic tumors. In this figure, the cases with p53 positive-lymph node metastatic tumors tended to be distributed in the right lower field ; this distribution pattern indicates that the lymph node metastatic tumors that were positive for p53 expression had higher proliferative activity and fewer cells undergoing apoptosis. In addition, there was a significant inverse correlation between apoptotic LIs and PCNA LIs ($r=-0.586$, $p<0.005$).

effects of mutated p53 protein in the tumor progression of esophageal carcinoma have not been reported clearly, since overexpression of p53 protein is not alway correlated with the clinicopathologic features or clinical outcome of esophageal cancer patients.

The clinical significance and/or prognostic value of p53 protein in esophageal cancer patients is under discussion. However, investigations about the mutations or overexpression of p53 protein performed thus far have been limited to primary tumors, so the molecular genetic events in lymph node metastatic tumors contributing to the malignant development of esophageal carcinoma have remained unclear.

In the present study, p53 overexpression in primary tumors did not necessarily reflect the actual progress of malignant development, hence overexpression of p53 protein may be an early event in esophageal carcinogenesis, as reported by Bennett et al.[5] However, in lymph node-positive cases, the incidence of p53 overexpression in cases with distant lymph node metastasis was significantly higher than that in cases without distant lymph node metastasis, and p53 overexpression in lymph node metastatic tumors was significantly related to a poor prognosis. Furthermore, our results showed that p53 overexpression in lymph node metastatic tumors can be a useful independent prognostic factor for lymph node-positive esophageal cancer patients. Therefore, p53 overexpression may contribute to malignant development of esophageal carcinoma after the cancer cells have metastasized to lymph nodes. In addition, in comparison of

the immunoreactivity for p53 between primary tumors and lymph node metastatic tumors, the cancer cells of lymph node metastatic tumors were stained diffusely, although primary tumors of the same cases demonstrated a heterogeneous staining pattern or were absent for p53 except for one case. These results of p53 immunostaining indicate that a small number of p53-positive cancer cells may be selected in the process of metastasis and proliferation in metastatic lymph nodes, resulting in contribution to malignant development of esophageal carcinomas, even if they are not initially detectable immunohistochemically in primary tumors. In the case with a p53-positive primary tumor and a p53-negative lymph node metastatic tumor, additional investigations may be needed to determine whether cancer cells in primary tumor acquired p53 overexpression after metastasis, or whether the p53-negative cancer cells acquired a higher metastatic potential due to the other genetic alterations.

In the present study, we also investigated the relationship between p53 overexpression and cell kinetics in primary and lymph node metastatic tumors. The positive correlation between p53 overexpression and proliferative activity has been reported in various tumors by other researchers.[28,29] There are various markers to estimate the proliferation rate of tumors. The proliferating cell nuclear antigen (PCNA) that we used is one such marker of cell proliferation, and expression of this antigen is closely linked to the cell cycle; the levels of PCNA increase during the late G1 phase immediately before the onset of DNA synthesis, reach maximum level during the S phase, decline during the G2-M phase, and are absent in quiescent cells during the G0 and long G1 phases.[14-17] The PCNA is a 36 kd intranuclear polypeptide antigen known as an auxiliary protein of DNA polymerases.[16,17] The immunohistochemical study for this antigen can be performed using formalin-fixed, paraffin-embedded tissues, and allows retrospective evaluation. Recent studies showed that wild-type p53 protein inhibits cycle progression to the S phase, and that inhibition of cell cycle progression to this phase following wild-type p53 gene induction is accompanied by selective down-regulation of PCNA mRNA and protein expression.[30,31] In this study, the PCNA LI of lymph node metastatic tumors was significantly higher than that of primary tumors, and further, p53-positive lymph node metastatic tumors had significantly higher proliferative activity than p53-negative tumors. These results suggest that p53 overexpression may be related to the activation of cell proliferation in lymph node metastatic tumors of esophageal carcinoma, and may affect the clinical outcome of lymph node -positive esophageal cancer patients.

On the other hand, the relationship between p53 overexpression and apoptosis in human solid tumors has remained unclear. Recent studies revealed that cells from various human malignancies have a reduced ability to undergo apoptosis in response to some physiological stimuli, which may contribute to carcinogenesis, tumor growth, and metastasis.[32,33] Furthermore, the alterations in the control of apoptosis are considered to be as important as those of proliferaiton in the progression of cancer. Recently, it has become accepted that there are at least two distinct apoptotic pathways, one requiring p53 and one that is p53 independent.[34] A few researchers have reported that there was no significant correlation between p53 expression and apoptosis in esophageal carcinoma.[26,35] In contrast, another report indicated a significant inverse correlation between p53 mutation and apoptosis in esophageal cancer patients treated by chemotherapy and radiotherapy.[36] To evaluate the relationship between p53 expression and apoptosis in esophageal carcinoma, we used the in situ DNA nick end labeling method for

detecting apoptosis. The in situ visualizaiton technique for apoptotic nuclei that contain fragmented DNA was described by Gavrieli et al.[18] This method utilizes terminal transferase to incorporate biotinylated nucleotides, and the sensitivity of this method for detecting single apoptotic cells is theoretically far higher than that for characteristic DNA laddering in agarose gels, obtained by electrophoresis of extracted DNA. The reliability of this method has been confirmed by other researchers, although care must be taken in avoiding false-positive results since necrotic or degenerating tumor cells sometimes appear to give a similar staining pattern when formalin-fixed and paraffin-embedded sections are used.[21,22,25,26] In the present study, the apoptotic LI of lymph node metastatic tumors was significantly lower than that of primary tumors, and p53-positive lymph node metastatic tumors tended to show far less labeling of apoptotic cells than p53-negative tumors, although a significant difference was not found. A role for wild-type p53 as a regulator response to apoptotic stimulus such as DNA damage or hypoxia has been reported by some researchers.[32,37] However, normal developmental pathways to control apoptosis can operate independently of wild-type p53.[34] The weak effect of p53 overexpression on apoptotic LI shown in this study suggests that the spontaneous apoptotic pathway in esophageal carcinomas may be wild-type p53-independent, but may be up-regulated by p53.

The results of this study, showing that lymph node metastatic tumors with p53 overexpression had significantly higher proliferative activity and tended to have far fewer cells undergoing apoptosis, suggest that p53-positive cancer cells may characteristically have high proliferative activity resulting in proliferation of clonal subpopulations with high growth potential and inhibition of apoptosis in lymph node metastatic tumors. In contrast, primary tumors with p53 overexpression had higher labeling of apoptotic cells although they tended to have far higher proliferative activity. These findings indicate that induction of apoptosis in esophageal carcinomas may be related to a change in proliferative activity resulting in the selection of p53 -positive subpopulations with a high growth potential in the process of metastasis, and that cancer cells with p53 overexpression may proliferate clonally conquering the apoptotic stimulus from their surrounding microenvironment in metastatic lymph nodes. In conclusion, our results indicate that p53 overexpression is clearly related to tumor cell proliferation and down-regulation of apoptosis in lymph node metastatic tumors, and that p53 overexpression in lymph node metastatic tumors may correlate with the malignant potential of esophageal carcinoma.

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