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Significance of p53 protein expression in growth pattern of esophageal squamous cell carcinoma

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Abstract. The significance of intraepithelial carcinoma concomitant with esophageal squamous cell carcinoma during carcinogenesis and progression of the tumor has been discussed diversely. The purpose of the current study was to elucidate the relation between p53 protein expression and the growth pattern of the squamous cell carcinoma of the esophagus with attention to coexistence of intraepithelial carcinoma. Seventy cases with squamous cell carcinoma of the esophagus surgically resected without preoperative adjuvant therapy, including 49 cases with intraepithelial carcinoma contiguous to the invasive lesion, were analyzed immunohistochemically for p53 expression. Positive immunoreactivity of p53 was found in 36 (51.4%) of 70 cases. The frequency of p53 protein expression in cases with intraepithelial carcinoma (65.3%; 32/49) was significantly higher than that (19.0%; 4/21) in cases without intraepithelial carcinoma ($p<0.001$). The value of invasion coefficient, which indicates a ratio of the area of invasive cancerous lesion occupied in the whole lesion, in the cases with p53 protein expression was significantly smaller than that in the cases without p53 protein expression ($p<0.001$). In conclusion, p53 protein expression was found to be significantly related to the coexistence and spreading of intraepithelial carcinoma contiguous to squamous cell carcinoma of the esophagus.

Introduction

Regarding the significance of intraepithelial carcinoma concomitant with squamous cell carcinoma of the esophagus, a variety of investigations have already been made. We also previously reported that in squamous cell carcinoma of the esophagus, the more the main cancerous lesion progressed, the less frequently the incidence of intraepithelial carcinoma concomitant with the invasive lesion occurred, which indicated that, in squamous cell carcinomas of the esophagus, the intraepithelial components may originate from field carcinomatous transformation rather than from an intraepithelial spread from the main lesions, and also lent support to the concept of field carcinogenesis in the esophagus (1,2).

On the other hand, Mandard *et al* (3) and Soga *et al* (4) stressed with the histopathological investigations that intraepithelial carcinomatous lesions concomitant with esophageal squamous cell carcinoma were the results of intraepithelial spread from the invasive carcinoma. However, no biochemical or genetical investigations have been reported regarding this phenomenon in carcinomas of the esophagus.

The mutation of p53 gene is the most common genetic abnormality in human cancers (5,6), and the genetic alterations have been reported to be considered as an early event in carcinomas of the esophagus (7,8), head and neck (9,10), or lung (11,12), on the other hand, a late event in carcinomas of the colon (13), liver (14), or brain (15), during the multistage of the carcinogenesis. And it has been reported to be closely related to the progression or invasion in adenocarcinoma of colon and rectum (16), transitional cell carcinoma of urinary bladder (17), hepatocellular carcinoma (18), and oral squamous cell carcinoma (19).

In the current study, we evaluated the relation between p53 protein expression and intraepithelial carcinoma which is contiguously concomitant with invasive squamous cell carcinoma of the esophagus to observe the significance of p53 protein expression in the growth pattern of squamous cell carcinoma of the esophagus.

Materials and methods

Subjects. Seventy cases of esophageal squamous cell carcinoma, surgically resected without preoperative adjuvant therapy in the Second Department of Surgery, Kyushu University Hospital, were investigated in this study. Of these cases, 49 coexisted with intraepithelial carcinoma contiguous to invasive cancerous lesions, and the other 21 did not.

Histopathologic investigations. The resected specimens were fixed in 10% formalin, cut into 4-mm thick pieces, and then embedded in paraffin. For all sections, hematoxylin and eosin staining was done and histopathologic investigation was performed.

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Key words: esophageal carcinoma, p53 expression, intraepithelial carcinoma, growth pattern

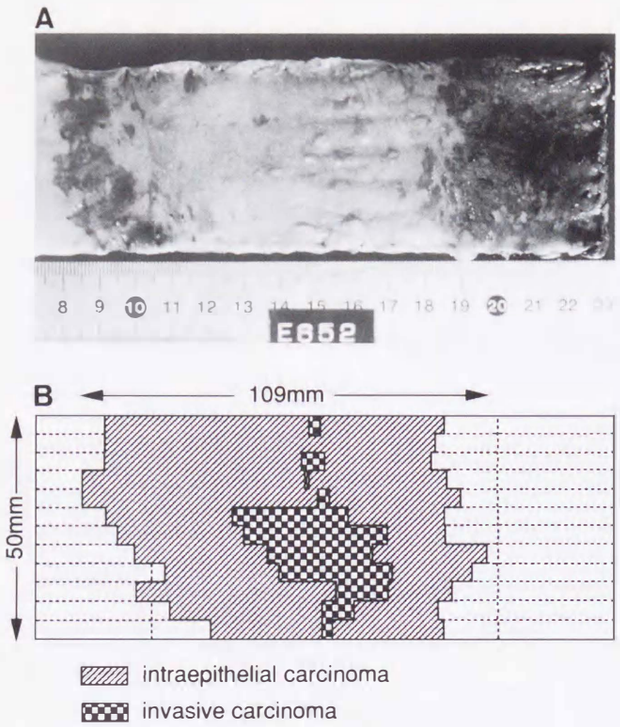

Figure 1. Culculation of invasion coefficient (IC). A, The resected specimen of a reprensentative case of esophageal carcinoma. The cancerous lesion was clearly observed after applying Lugol's solution. B, The map of lesions with esophageal carcinoma was drawn as a ground figure. The area of cancerous lesion was calculated as a geometrical value. In this case, the value of area of invasive lesions was 910 mm^2 as the sum of areas occupied by the invasive cancerous lesion, and the value of area of the entire cancerous lesion was 4,970 mm^2, the invasion coefficient (IC) of this case was 18.3%.

Table I. Clinicopathological factors of esophageal carcinomas with regard to p53 protein expression.

Clinicopathological factors	p53 protein expression: Positive (n=36)	p53 protein expression: Negative (n=34)	p-value
Location of tumors			
Upper	5	2	
Middle	18	23	NS
Lower	13	9	
Differentiation			
Well	2	3	
Moderately	23	19	NS
Poorly	11	12	
Depth of invasion			
muscularis mucosa	4	0	
submucosa	16	13	NS
muscularis propria	8	5	
adventitia	8	16	
Lymph node metstasis			
Negative	18	19	NS
Positive	18	15	
Lymphatic invasion			
Negative	17	22	NS
Positive	19	12	
Venous invasion			
Negative	25	24	NS
Positive	11	10	

NS, not significant.

Criteria of intraepithelial carcinoma. To diagnose intraepithelial carcinoma, we used the criteria of Suckow *et al* (20): i) absence of cellular differentiation with variations in size and shape, and hyperchromatism of the nuclei with increased mitotic activity; ii) the aforementioned changes must involve the entire thickness of the epithelium and may involve submucous grands and ducts; and iii) intact basement membrane. Excluding cases of multiple intraepithelial carcinoma, carcinomatous lesions associated with intraepithelial portions of continuous malignant changes were analyzed.

Immunohistochemistry. For p53 immunostaining, the avidin-biotin-peroxidase complex method was used. 4 μm-thick sections sliced from paraffin-embedded specimen were prepared on the slideglass pre-coated with poly-L-lysin. PAb 1801 (Ab-2; Oncogene science, Inc., Manhasset, NY), mouse monoclonal p53 antibody, which detects both wild and mutant types of human p53 protein, was used in this study. After removing paraffin in xylene, washing in ethanol inactivating endogenous peroxidase activity in methanol with 0.3% H_2O_2, exposure to microwaves for 20 min, and blocking cross-reactivity with the preimmune 10% rabbit serum (Histofine SAB-PO (M) Kit; Nichirei Co., Tokyo, Japan) for 30 min, the samples were incubated with primary antibody in a moist chamber overnight. After washing in phosphate-buffered saline (PBS), biotinylated rabbit anti-mouse immunoglobulin was applied, and then the samples were incubated in a moist chamber for 30 min at room temparature. After washing in PBS, the samples were incubated with peroxidase-conjugated streptavidin for 30 min at room temparature. After washing in PBS, the localization of p53 protein was visualized with diaminobenzidine tetrahydrochloride.

Culculation of invasion coefficient. From the histopathologic investigation of hematoxylin-eosin staining, the lesions of subjective cases with esophageal squamous cell carcinomas were drawn as ground figures (Fig. 1). The cancerous lesions which progressed over epithelium of the esophagus were treated as invasive lesions. Then the invasion coefficient (IC), which is considered to be an indicator reflecting the spread of intraepithelial carcinoma, was calculated as a proportion of area occupied by the invasive lesion for all the cancerous lesions (IC = area of invasive lesion/area of whole cancerous lesions).

Table II. p53 protein expression in esophageal carcinomas with contiguous intraepithelial carcinoma.

		Intraepithelial carcinoma		
		p53 (+)	p53 (-)	Total
Invasive carcinoma	p53 (+)	32	0	32
	p53 (-)	0	17	17
Total		32	17	49

p53 (+), p53 positive; p53 (-), p53 negative.

Table III. p53 protein expression and coexistence of contiguous intraepithelial carcinoma.

Coexistence of intra-epithelial carcinoma	p53 protein expression		Total
	Positive (36 cases)	Negative (34 cases)	
Positive (%)	32 (65.3)[a]	17 (34.7)	49 (100)
Negative (%)	4 (19.0)[a]	17 (81.0)	21 (100)
Total	36	34	70

Values in parentheses are percentages. [a]p<0.001.

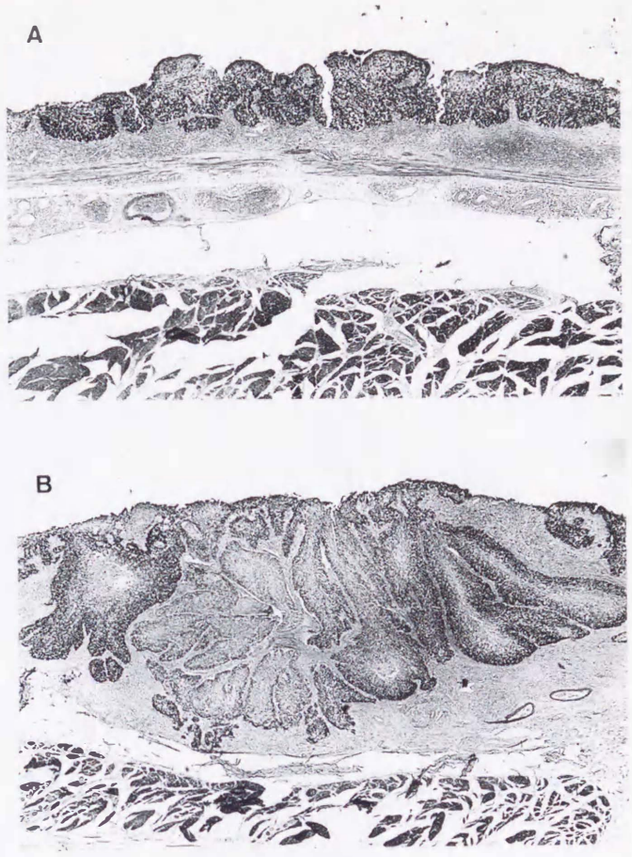

Figure 2. p53 immunohistostaining of a reprensentative case of esophageal squamous cell carcinoma. A, p53 protein expression in intraepithelial carcinoma contiguously concomitant with main invasive carcinoma (H&E, original magnification x42). B, p53 protein expression in an invasive carcinoma (H&E, original magnification x42). In both lesions p53 protein was localized only in the nuclei of the cancer cells.

Statistical analysis. Chi-square test was used to investigate the relations between p53 protein expression and the coexistence of intraepithelial carcinoma concomitant with carcinoma of the esophagus as well as between p53 protein expression and the clinicopathologic features. Mann-Whitney test was used to compare the data of IC. Any differences with a p-values of lower than 0.05 were considered to be significant.

Results

Relationship between p53 protein expression and clinicopathologic backgrounds. Among 70 cases with esophageal squamous cell carcinoma, 36 (51.4%) showed positive immunoreactivity. There was no statistical differences between cases with and without p53 protein expression relating to clinicopathologic features (Table I).

p53 protein expression. Among 49 cases coexisting with intraepithelial carcinoma, 32 had p53 protein expression in both intraepithelial and invasive carcinoma, and in the other 17 cases, p53 protein expression was found in neither intraepithelial carcinoma nor invasive cancerous lesion. In addition, among cases with contiguous intraepithelial carcinoma there was no case with different imunoreactivity in intraepithelial and invasive carcinomas (Table II). The immunohistochemical findings of a representative case with intraepithelial carcinoma contiguously concomitant with invasive lesion of esophageal squamous cell carcinoma are demonstrated in Fig. 2. In both lesions p53 protein was localized only in the nuclei of cancer cells.

Relationship between p53 protein expression and the coexistence of intraepithelial carcinoma. In the 49 cases coexisting with intraepithelial carcinoma contiguous to invasive cancerous lesions, 32 (65.3%) had p53 protein expression in both the intraepithelial carcinoma and the invasive cancerous lesion. On the other hand, in the 21 cases without intraepithelial carcinoma contiguos to invasive cancerous lesion, only four (19.0%) demonstrated p53 protein expression. There was a significant statistical difference between these proportions (p<0.001) (Table III).

Relationship between p53 protein expression and invasion coefficient. In 21 cases without intraepithelial carcinoma contiguously concomitant with invasive lesion, all values of IC were treated as 100%. In the 34 cases without p53 protein expression, the mean IC was 88.7±19.1%. On the other hand, in 36 cases with p53 protein expression, the mean IC was 53.4±28.2%, and the proportion was smaller than that of the former 34 cases without p53 protein expression

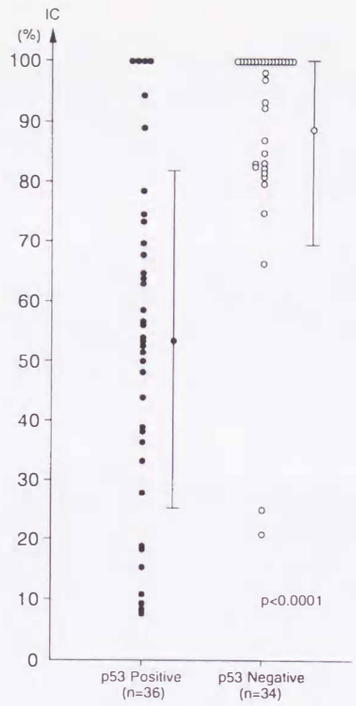

Figure 3. The relation between p53 protein expression and invasion coefficient (IC). The mean IC in 36 cases with p53 protein expression was 53.4±28.2%, which was significantly smaller than that (88.7±19.1%) in 34 cases without p53 protein expression (p<0.0001).

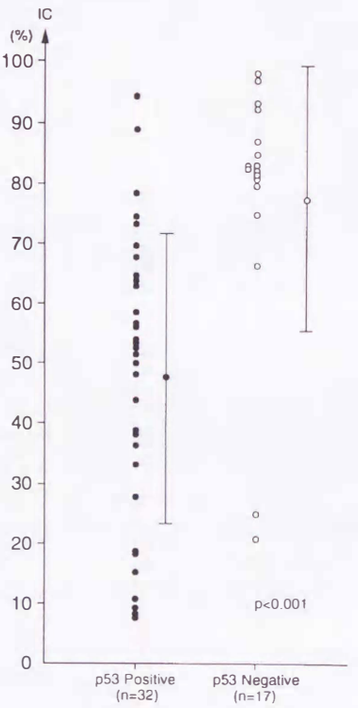

Figure 4. The relation between p53 protein accumulation and invasion coefficient (IC) among cases with intraepithelial carcinoma contiguosly concomitant with invasive carcinoma. The mean IC in 32 cases with p53 protein accumulation was 47.6±24.2%, which was significantly smaller than that (77.3±21.9%) in 17 cases without p53 protein accumulation (p<0.001).

(p<0.0001) (Fig. 3). In the investigation restricted to cases with intraepithelial carcinoma contiguosly concomitant with invasive lesion, the mean IC (47.6±24.2%) in 32 cases with p53 protein accumulation was also significantly smaller than the mean IC (77.3±21.9%) in 17 cases without p53 protein expression (p<0.001) (Fig. 4).

Discussion

The existence of intraepithelial carcinoma contiguously concomitant with invasive cancerous lesion has been reported to be a relatively frequent event in the squamous cell carcinoma of the esophagus (1-4).

The significnce of p53 gene alterations in squamous cell carcinoma of the esophagus remains controversial. Benett *et al* (8) reported that the mutations of p53 gene occur in esophageal dysplasia and were concerned with the growth advantage of cancer cells in esophageal squamous cell carcinoma. Sarbia *et al* (21) suggested that there was no significant relation between the overexpression of p53 gene and the prognosis or clinicopathologic features of patients with esophageal carcinoma. The purpose of this study was to elucidate the relation between p53 protein expression and the growth pattern of the esophageal carcinoma with special attention to the coexistence of intraepithelial carcinoma.

In our study, the ratio of p53 protein expression in esophageal squamous cell carcinoma was 51.4% (36/70), which was generally consistent with the values in previous reports (22,23). The proportion (65.3%; 32/49) of cases with p53 protein expression in cases coexisting with intra-epithelial carcinoma contiguous to invasive cancerous lesion was found to be significantly larger than that (19.0%; 4/21) in the cases without intraepithelial carcinoma. These data thus suggested that p53 protein expression is considered to be closely related to the formation of intraepithelial carcinoma contiguously concomitant with squamous cell carcinoma of the esophagus. Moreover the IC was adopted to investigate the relation between p53 protein expression and growth pattern of esophageal squamous cell carcinoma. The significance of IC expressed that carcinomas with a larger IC value had a trend of profound invasion, while on the other hand, carcinomas with smaller IC value had a trend of intraepithelial spread. The value of the IC in cases with p53 protein expression was significantly smaller than that in cases without p53 protein expression. These data thus suggested that squamous cell carcinomas of the esophagus with p53 protein expression have significant relation to the intraepithelial spreading type growth, while carcinomas without p53 expression have significant relation to the profound penetrating type growth.

Concerning the relation between p53 protein expression and growth pattern of carcinoma, Oiwa *et al* (24) reported that there was a significant relation between p53 expression and the penetrating type growth pattern of gastric adeno-carcinomas. Our data showed that p53 expression was significantly related to intra-epithelial spreading type growth of esophageal squamous cell carcinoma. The functions of p53 protein on the growth of carcinomas have not been fully elucidated, however, it may mediate the growth pattern of squamous cell carcinoma of the esophagus.

In conclusion, p53 protein expression was found to be significantly related to the coexistence and spread of intraepithelial carcinoma contiguous to squamous cell carcinoma of the esophagus and it is thus considered that the intraepithelial spreading type growth of squamous cell carcinoma of the esophagus may be potentially mediated by p53 mutation.

Acknowledgments

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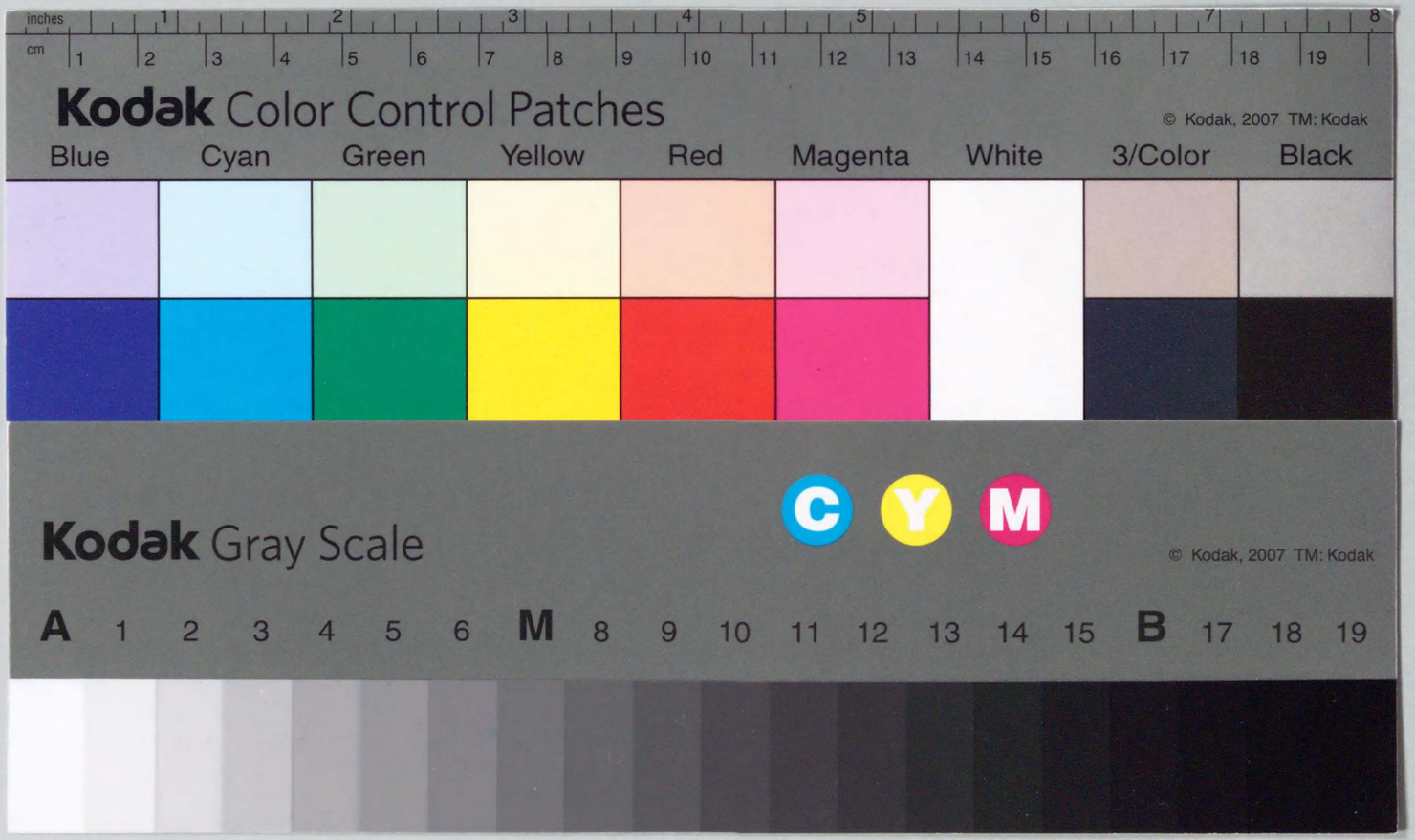
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