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Biological Behavior of Mucoepidermoid Carcinoma of the Esophagus

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Abstract

Mucoepidermoid carcinoma of the esophagus (MEC) is uncommon and has not been fully investigated. The biological behavior and clinical aspects of MEC were studied.

The clinical features of eight patients with MEC were compared with 51 cases of squamous cell carcinoma of the esophagus (SCC). Proliferating cell nuclear antigen (PCNA), p53, and carcinoembryonic antigen (CEA) were stained in the resected specimens by immunohistochemistry

Seven out of 8 cases (87.5%) had stage III by TNM classification. Four cases died of widespread metastases and 2 cases died of local recurrence within 2 years after the surgery. Neither chemotherapy and radiotherapy were effective against MEC. Overall median survival periods were 10.8 months for MEC and 32.1 months for SCC ($P<0.05$). When patients in stage III alone were compared, MEC tended to have a worse prognosis than SCC ($P=0.058$). Immunohistochemical studies revealed that the positive rates of PCNA and CEA were significantly higher in MEC than in SCC ($P<0.05$), while there was no significant difference in p53 positive rate.

Esophageal MEC had an aggressive biological nature and was resistant to adjuvant therapies. The poor prognosis of esophageal MEC may be caused by high proliferative and metastatic potential. (J Nippon Med Sch 2003; 70: 401–407)

Key words: mucoepidermoid carcinoma, esophagus, prognosis, immunohistochemistry, p53

Introduction

Mucoepidermoid carcinoma (MEC) of the esophagus is known to be uncommon with an incidence of less than 1% of all malignant esophageal neoplasms[1,2]. Sixty-seven cases of MEC were identified in the literature including Japanese reports until December 2000[2–6]. Its biological behavior and response to therapies have not been well studied, but MEC appears to have a greater incidence of recurrence and distant metastasis regardless of treatments than those of squamous cell carcinoma (SCC)[3,5]. Recently, expression of proliferating cell nuclear antigen (PCNA) has been reported to increase in the late G1 and S phases of the cell cycle and has been used as a predictor of the cell proliferative state. In SCC of the esophagus, it has been reported that prolifera-

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tive activity estimated by PCNA and Ki-67 influences outcome[7,8]. Other studies suggest that PCNA immunostaining combined with argyrophilic nucleolar organizer region (AgNOR) or epidermal growth factor receptor (EGFR) is a good prognostic factor in SCC of the esophagus[9,10]. Mutations of the p53 suppressor gene are frequently found in gastrointestinal carcinomas including the esophagus[12]. In esophageal carcinoma, overexpression and/or accumulation of p 53 may be related to an unfavorable prognosis[13]. To clarify the relation between clinical evidence and pathomolecular properties of this uncommon tumor, we have investigated the the clinical characteristics, survival rate, and immunohistochemistry for PCNA, p53 and carcinoembryonic antigen (CEA) in 8 cases of MEC in comparison with 51 cases of SCC of the esophagus.

Subjects and Methods

1. Subjects

Two hundred and seventy-eight cases of esophageal carcinoma were resected in the Department of Surgery I, Nippon Medical School Hospital between 1978 and 1997. Of the 278 surgically treated patients, 252 patients (90.6%) had ordinary SCC. MEC was found in 8 (2.9%), adenocarcinoma in 10 (3.6%), and other tumors in the remaining 8 patients (2.9%). We previously reported 5 cases of MEC[4,6]. We defined mucoepidermoid carcinoma in this study as a tumor which contains an intimate mixture of carcinomatous squamous and mucin-secreting cells according to WHO classification of the esophageal tumors[11]. Each of the components accounted for at least 20% of the tumor. In addition to routine hematoxylin and eosin staining, periodic acid-Schiff, alcian blue, and mucicarmine stains were applied for detection of mucus in MEC. Clinical responses to chemotherapy and/or radiotherapy for carcinoma were defined according to the WHO criteria[14]. As summarized in **Table 1**, the age of patients with MEC ranged from 47 to 66 years with a mean age of 58 years, and all of them were male. Every patient with MEC underwent esophagectomy with lymph node dissection. Seven patients had stage III except 1 patient with stage IIA. Two cases also received a total of 60-gray of irradiation preoperatively, and the other 3 cases received preoperative chemotherapy of continuously infused 500 mg/m^2/day 5-fluorouracil (5-Fu) and 10 mg/m^2 of leucovorin every 12 hours on days 1 through 5 and 70 mg/m^2/day of cisplatin (CDDP) on day 5. The clinical, histologic, and immunohistochemical characteristics were compared in 51 consecutive patients (46 males and 5 females) with SCC undergoing esophagectomy with lymph node dissection between 1992 and 1997. Because preoperative chemotherapy for locally advanced SCC patients started in 1992 in our department, we chose the patients with SCC for comparisons. Ten of the patients with SCC received preoperative chemotherapy using same regimen. The age of the SCC patients ranged from 42 to 81 with a mean age of 61 years. Tumor staging of SCC was stage 0 in 3, stage I in 4, stage IIA in 6, stage IIB in 16, stage III in 21, and stage IV in 1 patient by TNM classification (UICC) (**Table 1**). This study was approved by the Nippon Medical School Hospital Ethical Committee. Informed consent was obtained from all patients before the treatments.

Table 1 Clinical characteristics of patients with mucoepidermoid carcinoma (MEC) and aquamous cell carcinoma (SCC)

	MEC	SCC
Number	8	51
Age (mean)	47—66 (58)	42—81 (61)
Male/Female	8/0	46/5
TNM stage		
0	0	3
I	0	4
IIA	1	6
IIB	0	16
III	7	21
IV	0	1

2. Immunohistochemistry

Resected specimens were fixed in 10% neutral formalin and embedded in paraffin blocks by conventional techniques. The entire specimen was cut and blocked at a thickness of 5 mm, and the paraffin blocks were sliced serially into 5 μm sections.

For PCNA staining, sections were deparaffinized

in xylene, dehydrated using graded ethanol concentrations, and washed in water. As a pretreatment, sections were immersed in distilled water in a thermoresistant plastic box and were processed in a microwave oven (700 W) for 10 minutes. Immunohistochemical staining was performed using a streptavidin-biotin staining technique (Histofine SAB-PO (M) kit, Nichirei, Tokyo, Japan). The sections were treated with 3% hydrogen peroxide for 15 minutes to block endogenous peroxidase activity, and incubated with 10% normal rabbit serum to block nonspecific antibody binding. Next, they were incubated with PCNA monoclonal antibody PC-10 (DAKO, Glostrup, Denmark), diluted 1: 100 at room temperature for 2 hours, and then incubated with biotinylated rabbit anti-mouse IgG + IgA + IgM for 10 minutes. Finally, they were incubated with peroxidase streptavidin for 30 minutes. Between each step, the sections were washed three times in phosphate buffer solution for 5 minutes. Diaminobenzidine-hydrogen peroxidase was used as a chromogen, and Mayer's hemotoxylin stain was used as a counterstain.

For p53 staining, as a pretreatment, sections were placed in 10 mM citric acid monohydrate buffer (pH 6) and were boiled under a pressure of 2 ATM for 2 minutes. Then they were treated in the same procedure as PCNA staining. p53 monoclonal antibody DO7 (Novocastra, Newcastle, UK) diluted 1: 100 was used as the primary antibody. For CEA staining, the same method as for PCNA staining was used without microwave, and with CEA monoclonal antibody (Immunotech, Marseille, France) used as the primary antibody.

3. Assessment of staining

PCNA staining: Cells were considered positive for PCNA when an unequivocal diffuse or dot-like brown nuclear staining could be identified. The photomicrographs were taken from each section stained for PCNA in a randomly selected area and individual positive cells were counted. Positive indices for PCNA were calculated as percentage values taking the total number of examined cells into account. At least 1,000 carcinoma cells were counted for each section. The percentage of carcinoma cells with nuclei stained for PCNA, called the PCNA labeling index (LI), was calculated for each section.

Table 2 Immunohistochemical expression of p53 and CEA in esophageal MEC and SCC

	p53 positive	CEA positive
MEC	5/8 (63%)	8/8 (100%)
	P = 0.45	P = 0.006
SCC	27/51 (53%)	25/51 (49%)

p53 staining: Tissue sections were evaluated for cells expressing p53 in the nuclei without cytoplasmic staining. Specimens in which over 10% of cancer cells were immunostained with p53 were classified as positive. Furthermore, patients with SCC were divided into positive and negative groups according to the results of p53 staining, and survival rates were analyzed between the two groups.

CEA staining: Tissue sections in which staining was observed in the membrane or cytoplasm of the cancer cell nests were determined as positive. When the staining was partly positive for the keratin layer in the normal epithelium, the well keratinized area of the central of the cancer nest, and the necrotic area in the tumor, we judged them as not having positive expression.

Immunohistochemical expression was examined statistically between MEC (n = 8) and SCC with stage III (n = 51).

4. Statistical Analysis

The PCNA indices were analyzed using the two-tailed Mann-Whitney U-test. Fisher's exact probability test was used to compare frequency of positive p53 and CEA. Survival rates were calculated by the Kaplan-Meier method, and the log-rank test was used to evaluate the statistical significance. A *P* value of 0.05 or less was considered statistically significant.

Results

1. Clinical and pathological features of MEC

The clinical data of the 8 patients with MEC are shown in **Table 2**. Seven patients (87%) had both smoking habit and high alcohol consumption. Dysphagia was found in 7 patients (87%). Tumors

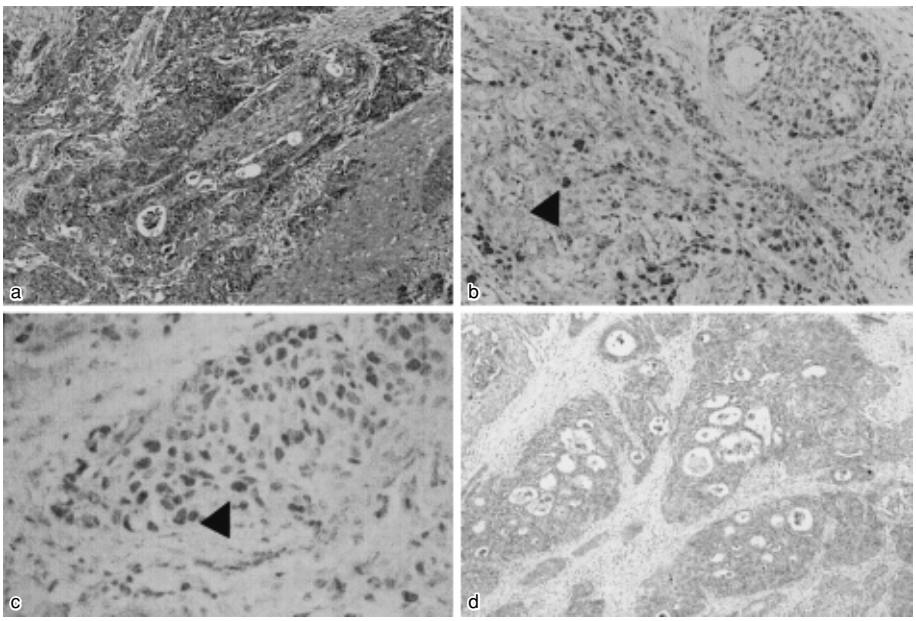

Fig. 1 Microphotograph revealing mucoepidermoid carcinoma (MEC) consisting of a diffuse mixture of squamous and mucin-secreting carcinoma cells (a: HE stain, ×100). Positive stains are found in the nuclei of the carcinoma cells with PCNA staining (b) and p53 staining (c). (×200) (arrow) Positive CEA staining is localized in the membranes and cytoplasm of the mucous-containing adenocarcinomatous cells in MEC (d). (×25)

were located in the middle (50%), middle to lower (38%), and upper to middle (12%) portions of the esophagus. Gross features were divided into indurated type in 6 (75%) and ulcerative type in 2 (25%). Five tumors intimately had a mixture of glandular and squamous patterns (case 3, 4, 5, 6, 7) (**Fig. 1a**), and 3 tumors contained squamous carcinoma cells and carcinomatous signet-ring cells (cases 1, 2, 8). Tumors invaded the adventitia of the esophagus in 7 patients (87.5%) and the right main bronchus in the remaining patient (12.5%). Invasion to both lymph and blood vessels was observed in 7 (87.5%), and lymph node metastases were also found in 7 patients.

Esophagograms demonstrated funnel-shaped stenosis of the esophagus in 3 (37.5%), irregular stenosis in 4 (50%), and a protruding mass in 1 patient (12.5%). Endoscopic examination revealed common characteristics in which elevated tumors were mostly covered by normal epithelia on the adoral side with or without shallow ulceration. Covering epithelia on tumors were often positively stained by iodine.

Chemotherapy was given to cases 5, 6 and 8 and radiotherapy to cases 2 and 3 before operation. However, none of the cases had a decrease of over 50% in the tumor size, and they were estimated as no change (NC). On the other hand, 8 of 10 patients with SCC were estimated to be partial response (PR).

2. Prognosis of patients with MEC and SCC

Recurrence was found in the mediastinum in 33% (2/6) of the patients who died of MEC. Metastases to the lung, liver, brain, bone and/or peritoneum were found in 67% (4/6) within 12 months after surgery. Lung metastasis was the most common. In contrast to MEC, mediastinal recurrence was found more frequently in the patients with SCC. When case 2, who died of postoperative renal failure, was excluded, the overall median survival of MEC was 10.8 months after operation (range 4 to 24), while that of SCC was 32.1 months (range 3 to 70). There was a significant difference in Kaplan-Meier survival between the two groups ($P = 0.043$) (**Fig. 2a**). When the patients with stage III alone were selected, there was no significant difference between MEC and SCC. However, the patients with MEC tended to have a worse prog-

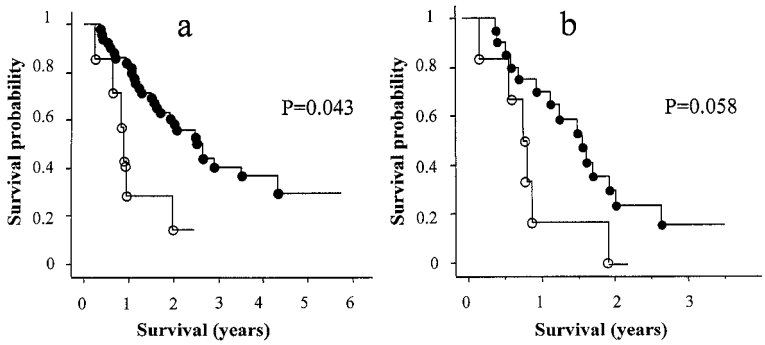

Fig. 2 (a) Overall Kaplan-Meier survival curves of patients with mucoepidermoid carcinoma (MEC : ○) and squamous cell carcinoma (SCC : ●) showing significant difference in survival (P = 0.043).
(b) Survival curves of patients with MEC (○) limited to stage III show a tendency for a worse prognosis those that with SCC (●) (P = 0.058).

nosis than those with SCC (P = 0.058) (**Fig. 2b**). In our series of 51 SCC patients, there was no correlation between immunohistochemical expression of p 53 and prognosis (P = 0.875).

3. Immunohistochemistry

Positive staining for PCNA was found in the nuclei of the carcinoma cells (**Fig. 1b**). The mean ± standard deviation (SD) of PCNA LI was 50.6 ± 26.2 % in MEC (n = 8), and 30.4 ± 16.7% in SCC (n = 51) (P = 0.032) (**Fig. 3**). p53 immunoreactivity was not seen in intact esophageal mucosa, but was found in the nuclei of carcinoma cells (**Fig. 1c**). There was no significant difference in positivity of p53 between MEC (5/8; 63%) and SCC (27/51; 53%) (p = 0.45) (**Table 2**). Positive CEA immunostaining was localized in the cell membranes and in the cytoplasm of the mucous-containing adenocarcinomatous cells in MEC (Fig. 1d). Positive staining for CEA was seen in all cases with MEC, and in 25 of 51 cases (49%) with SCC (p = 0.006) (**Table 2**).

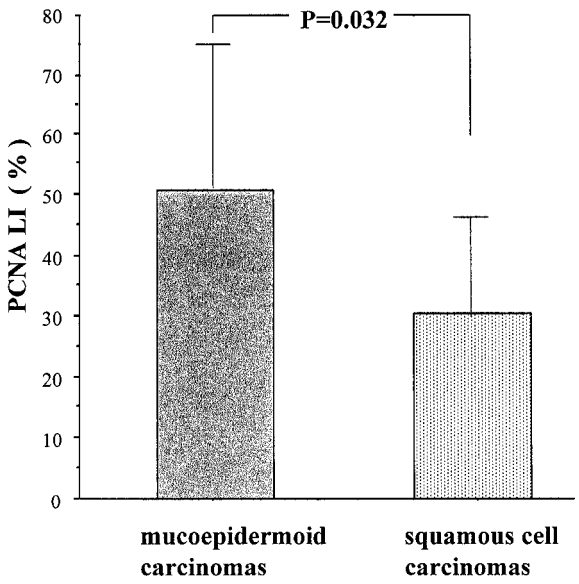

Fig. 3 Expression of proliferating cell nuclear antigen (PCNA). Positive rate for PCNA staining was significantly higher in mucoepidermoid carcinomas (MEC) than in squamous cell carcinomas (SCC) (P = 0.032).

Discussion

Our 8 patients presented clinical and pathologic features that were common to the 66 reported cases[2-6]. MEC was characterized by its indurated or ulcerated appearance on gross examination. The tumors were mostly covered by normal epithelia, although endoscopic findings revealed the tumor had extensively invaded the esophageal wall[4]. Most of them were men and over 50 years of age. The tumor was located mainly in the middle and lower thirds of the esophagus. Approximately, 80% of the tumor invaded the adventitia and metastasized to the lymph nodes. Although most patients underwent surgical resection and/or irradiation, the prognosis was poor. More than 60% of the patients died as a consequence of widespread metastases within one year after surgery. Only 8 patients survived more than 2 years. These results may reflect the unique

histogenesis of this tumor. Since MEC has been reported to arise from the esophageal gland and its ductal epithelium, MEC may invade the esophageal wall deeply and patients would be asymptomatic in the early phase of the development[3].

Most primary MEC in the salivary glands is believed to be low grade malignancy with lesser metastatic tendency[15], whereas, primary MEC of the esophagus has been reported to have high grade malignancy[3]. However, it is not clear whether MEC of the esophagus is more progressive than SCC. In the present study, there was a significant difference in overall Kaplan-Meier survival between 8 patients with MEC and 51 patients with SCC. Although the number of patients with MEC was small, MEC tended to have poorer prognosis than SCC when the patients with stage III alone were selected.

It was also reported that MEC of the esophagus had lower sensitivity to chemotherapy or radiotherapy than SCC[5]. Lieberman et al. reported that only 2 out of 14 cases survived more than 2 years after surgery followed by adjuvant chemo-radiotherapy[2]. Preoperative chemotherapy using CDDP and 5-Fu has been very effective in patients with SCC[16,17]. Our 5 cases of MEC received preoperative radiotherapy or chemotherapy, but this had little effect in endoscopic and esophagographic assessments, suggesting that neither radiotherapy nor chemotherapy may be effective for MEC. Hematogenic metastasis to the distant organs was most common in MEC. Therefore, the poor prognosis of MEC is caused by both the highly metastatic potential and resistance to various therapies.

An immunohistochemical study of the tumor-associated antigens and proteins of oncogenes or tumor suppressor genes reveals the biological nature of the tumors and frequently predicts the progress of the tumors[7–10]. In this study, we stained p53, PCNA, and CEA to clarify the tumor suppressor gene alterations, cell proliferative activity, and the biological behaviors. p53 gene mutations were frequently found in gastrointestinal carcinomas including esophageal carcinoma[12]. However, the correlation between immunohistochemical p53 expression and prognosis of the esophageal carcinoma is controversial[13,18]. In our series of 51 SCC patients, there was no correlation between immunohistochemical expression of p53 and prognosis. Furthermore, there was no significant difference in the immunohistochemical positive rate for p53 between MEC and SCC. These results suggest that immunohistochemical expression of p53 was not related to the prognosis of the patients with esophageal carcinomas.

The expression level of PCNA is widely accepted as a good indicator of cell proliferation[19], it was reported that esophageal SCC patients with higher PCNA LI have poorer prognosis[7]. Because PCNA LI in MEC was significantly higher than in SCC in our study, MEC might have higher proliferative activity than SCC. With regard to the sensitivity to radiotherapy or chemotherapy, there are several reports showing that tumors with high PCNA LI are sensitive to chemotherapy or radiotherapy[20]. On the other hand, it is reported that low expression of PCNA showed good response to chemotherapy in esophageal carcinoma[21]. Further, there was a trend for downstaging by preoperative radiotherapy in rectal carcinoma with low PCNA LI[22]. Although it is still controversial, these reports support our findings that high PCNA LI may be resistant to chemotherapy and/or radiotherapy in MEC.

Immunostaining and serum levels of CEA were reported to predict metastasis or recurrence in patients with carcinoma including the esophagus[23,24]. It was reported that CEA-expressing adenocarcinoma cells could adhere to endothelial cells, thus facilitating hematogenic metastasis[25]. Sanders et al. also suggested that changes in subcellular distribution of CEA may be related to the spread and dissemination of SCC of the esophagus[26]. In this study, positive staining for CEA was found in all cases of the adenocarcinomatous components in MEC, while the positive rate was only 33% in SCC. Therefore, the expression of CEA possibly relates to the metastatic potential in MEC of the esophagus.

In conclusion, MEC of the esophagus showed aggressive biological characteristics and was resistant to adjuvant therapies. The poor prognosis of the patients with MEC may be caused by the high proliferative and metastatic potential, both of which were shown in this immunohistochemical study.

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