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Significance of Macrophage Chemoattractant Protein-1 Expression and Macrophage Infiltration in Squamous Cell Carcinoma of the Esophagus

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OBJECTIVES: Macrophage chemoattractant protein-1 (MCP-1) is a chemokine-inducing infiltration of macrophages, which can play several roles in tumor growth and metastasis. We have attempted to clarify the relationship between MCP-1 expression and macrophage infiltration in esophageal squamous cell carcinoma (SCC).

METHODS: Paraffin-embedded sections of tissue samples taken from 56 patients with esophageal SCC after curative surgery were immunohistochemically stained for MCP-1, CC chemokine receptor 2 (CCR-2), and thymidine phosphorylase (TP). Macrophage recruitment in SCC was evaluated by monocytic count based on CD68 immunostaining. Microvessels immunostained for Factor VIII-related antigen were counted in SCC, and microvessel density (MVD) was determined. Ki-67 labeling index was calculated based on Ki-67 immunostaining, and an apoptotic index was calculated based on the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate biotin nick end labeling.

RESULTS: MCP-1 was expressed in cancer cells of 31 SCC (55.4%) and in stromal cells mainly identified as macrophages of 16 SCC (28.6%). CCR-2 was expressed in stromal cells of all SCC and in vascular endothelial cells of 15 SCC (26.8%). There was a significant correlation between the expression of MCP-1 in cancer cells and of CCR-2 in stromal cells. TP was expressed in stromal cells in 76.7% of the SCC. Monocytic count, MVD, and Ki-67 LI in SCC with MCP-1 expression in cancer cells were higher than that without, and apoptotic index in SCC with MCP-1 expression in cancer cells were lower than that without. Furthermore, the monocytic count was positively correlated with MVD, while it was inversely correlated with apoptotic index. Clinicopathologically, MCP-1 expression in cancer cells was correlated with venous invasion, distant metastasis, and lymph node metastasis. Monocytic count in SCC with venous invasion, distant metastasis, or lymph node metastasis was higher than that without them. Five-year survival rate in the patients with high monocytic count or MCP-1 expression was worse than that with a low monocytic count or without MCP-1 expression.

CONCLUSIONS: These results suggest that MCP-1 expression and macrophage infiltration is associated with angiogenic promotion in esophageal SCC. MCP-1 expression may be interactively associated with macrophage infiltration in esophageal SCC; MCP-1 may play an important role in tumor angiogenesis through production of angiogenic factors, such as TP, by recruited macrophages in esophageal SCC. Furthermore, CCR-2 expression in vascular endothelial cells may participate partially in angiogenesis. Clinicopathologically, esophageal SCC patients with MCP-1 expression have no favorable prognosis.

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INTRODUCTION

Angiogenesis in tumor stroma plays a key role in tumor growth, invasion, and metastasis, and is stimulated by several angiogenic factors and cytokines releasing from tumor cells and/or stromal cells (1, 2). Furthermore, interaction between tumor and stromal cells is essential for angiogenesis and tumor growth. Some of these stromal cells have been identified as tumor-associated macrophages, and they play an important role in tumor growth, invasion, and metastasis (3). Infiltrating macrophages in tumor stroma are regulated by several kinds of cytokines and growth factors (4, 5), including macrophage

chemoattractant protein-1 (MCP-1) (6). MCP-1 is a member of C-C chemokines (6), and possesses chemotaxic activity for macrophages (7). MCP-1 has been reported to be upregulated in several tumors (8, 9). Furthermore, MCP-1 may be an indicator of angiogenesis because MCP-1 gene transfection in tumor cells promotes angiogenesis, and MCP-1 transfection induces angiogenesis (10). In addition, MCP-1 may also have a direct effect on tumor angiogenesis by inducing chemotaxis of endothelial cells, as evidenced by the detection of CC chemokine receptor-2 (CCR-2), which is a receptor for MCP-1 in these cells. Thymidine phosphorylase (TP) is an angiogenic factor that is identified as platelet-derived endothelial cell growth factor (11, 12). In several tumors, such as breast cancer (13), TP was expressed in tumor-associated macrophages, and TP expression participates in tumor angiogenesis and metastasis. On the other hand, angiogenesis influences proliferation and apoptosis in tumors. However, it is not yet well known how MCP-1, which may be an angiogenic promoter, influences apoptosis and cell proliferation in tumors.

In squamous cell carcinoma (SCC) of the esophagus, it has already been reported that vascular endothelial cell growth factor (VEGF) (14) and TP (15) are major components of angiogenic factors, and their expression is associated with angiogenesis, a high incidence of distant metastasis, and a poor progonosis of patients. In the present study, MCP-1 expression in esophageal SCC was immunohistochemically examined, and the significance of MCP-1 expression and macrophage infiltration as well as the relationship between them were investigated in esophageal SCC. Furthermore, the relationship between TP and MCP-1 expression, and the relationship between MCP-1 expression and apoptosis/cell proliferation were investigated in esophageal SCC.

MATERIALS AND METHODS

Patients and Tissue Preparation

Tissue samples of esophageal SCC were obtained from 56 patients who underwent esophagectomy with curative intent, without having received preoperative chemotherapy and radiotherapy, between 1989 and 1998 in the Department of Surgery, Shinshu University Hospital. These tissues were fixed in 10% formalin buffered with phosphate at pH 7.4 and embedded in paraffin. Serial sections were made and mounted on poly L-lysine-coated glass slides. Routine histopathological examination was performed to determine histological differentiation, depth of invasion, presence of lymph node metastasis, and lymphatic and venous invasion according to the TNM classification (16).

Immunostaining for MCP-1, CCR-2, and TP

The avidin-biotin complex (ABC) method was used for MCP-1 immunostaining. The immunostaining was performed using anti-human MCP-1 monoclonal antibody (5D3-F7, Pharmingen, San Diego, CA) diluted 50-fold. Sections immersed in citrate buffer were treated in a microwave oven before the staining procedure. CCR-2 immunostaining was performed using anti-human CCR-2 monoclonal antibody (48607.121; Genzyme/Techne, MN) diluted 100-fold. TP immunostaining was performed using a mouse anti-TP IgG antibody (clone 654-1; Nippon Roche Research Center, Kamakura, Japan) (17) diluted 500-fold according to the ABC method. Visualization of the immunoreaction for MCP-1, CCR-2, and TP was performed with the staining medium for peroxidase containing 0.05% 3,3′-diaminobenzidine tetrachloride. For negative controls, nonimmunized mouse immunoglobulin-G was substituted for primary antibody at the same concentration as the test antibody in every run.

Expression of MCP-1 and TP in cancer or stromal cells was characterized as negative or positive, according to the extent of the immunostaining. If immunoreactivity of MCP-1 and TP was randomly expressed, it was judged to be positive when more than 10% of cancer cells were stained in each section. In well χ^2 and moderately differentiated SCC, the reaction was judged by excluding the keratinized portion localized at the central part of the cancer nest, called cancer pearl. MCP-1 and TP expression of stromal cells in SCC was judged to be positive if more than 10% of stromal cells were stained in each section.

Immunostaining and Count of Microvessels

Microvessels in SCC tissue were immunostained by the ABC method using rabbit antihuman factor VIII-related antigen polyclonal antibody (Dako, Glostrup, Denmark) diluted 300-fold. Microvessel count was performed according to the method of Maeda *et al.* (18). Briefly, the factor VIII-stained sections were screened at five-fold magnification under a microscope to identify the areas of highest vascular density within SCC tissue. Microvessels in cancer stroma were counted in the five areas with the highest density at 200-fold magnification. Microvessel density (MVD) was expressed as the average of the microvessel count in these areas.

Immunostaining for CD68 Antigen and Monocytic Count

Macrophages were identified by positive immunostaining for CD68 antigen. CD68 immunostaining was performed by the ABC method using monoclonal antibody KP1 (Dako Patts, Copenhagen, Denmark) diluted 100-fold. For counting macrophages in esophageal SCC, a method similar to that used to determine MVD was performed. After screening at low magnification under a microscope, five areas with a high density of CD68-positive stromal monocytes were selected, and CD68-positive stromal monocytes were counted at 200-fold magnification, and taken as the monocytic count.

Ki-67 Immunostaining and TUNEL

Ki-67 immunostaining was performed by the ABC method using anti-Ki-67 monoclonal antibody (MIB-1; Immunotech. S.A., Marseille, France) diluted 100-fold. Sections were treated in a microwave oven before the staining procedure. Visualization of the immunoreaction was performed with DAB.

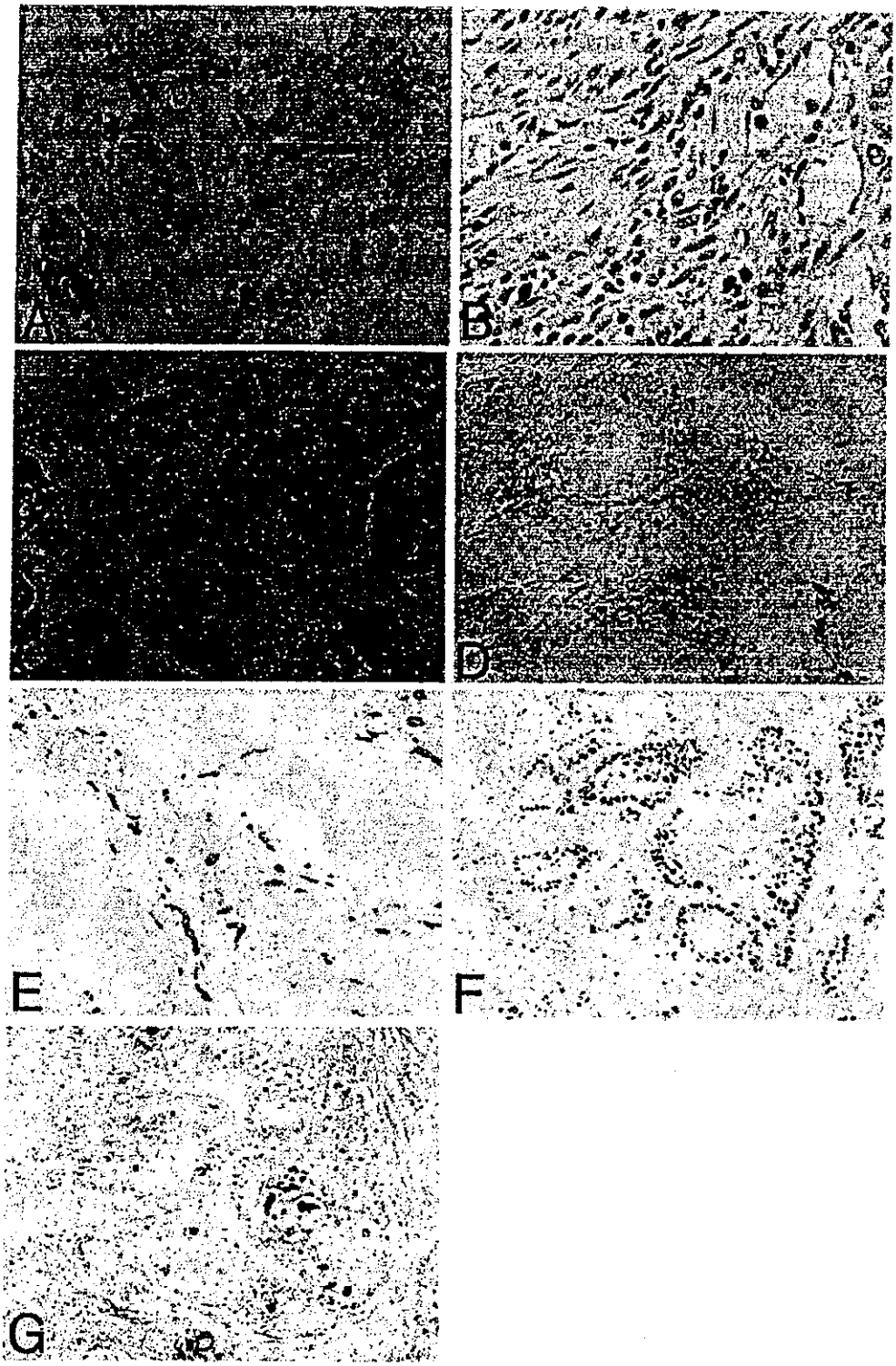

Figure 1. Immunohistochemical findings. (*A*) Macrophage chemoattractant protein-1 (MCP-1) immunostaining. Not only squamous cell carcinoma (SCC) cells but infiltrating stromal cells revealed a positive reaction for MCP-1. (*B*) CC Chemokine receptor-2 (CCR-2) immunostaining. Stromal cells revealed a positive reaction for CCR-2, as did vascular endothelial cells occasionally. (*C*) Tymidine phosphorylase (TP) immunostaining. TP was expressed in cancer and stromal cells. (*D*) CD 68 immunostaining. Stromal cells infiltrated in SCC mainly showed a positive reaction for CD 68. Most of these cells showed CD 68-positive reaction. (*E*) Factor VIII-related antigen immunostaining. A positive reaction for Factor VIII-related antigen is observed in the microvessels in the tumor stroma. (*F*) Ki-67 immunostaining. Ki-67-positive reaction is observed in the nuclei of the cancer cells. (*G*) TUNEL. A TUNEL-positive reaction is observed in the condensed nuclei of the cancer cells.

Table 1. Relationship Between MCP-1 and TP Expression of Cancer Cells and/or Stromal Cells

TP Expression in Stromal Cells	MCP-1 Expression					
	Cancer Cells			Stromal Cells		
	Positive	Negative	*p* Value	Positive	Negative	*p* Value
Positive	28	15	0.009	15	28	0.054
Negative	3	10		1	12	

MCP-1, macrophage chemoattractant protein-1; TP, thymidine phosphorylase.

A dark accumulation of DAB in the nuclei was judged to indicate a positive reaction for Ki-67. The percentage of cancer cells with nuclei stained for Ki-67, the Ki-67-labeling index, was calculated for each section on the basis of staining of about 2,000 cancer cell nuclei.

Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate–biotin nick end labeling (TUNEL) was performed according to the method of Gavrieli *et al.* (19), and briefly used subsequent reactions with proteinase K (20 mg/ml, Dako), and terminal deoxynucleotidyl transferase ($6.75 \times 10-2$ unit/ml, Life Sciences, St. Petersburg, FL) and biotinylated deoxyuridine triphosphate (3.75 pM/ml, Enzo Diagnosis, New York). The TUNEL reaction was visualized with DAB. Dark accumulation of DAB in the cells (nuclei and apoptotic bodies) was judged to indicate a positive reaction to TUNEL. When a few apoptotic bodies were clustered in a portion of a section, this aggregate was judged to have originated from an apoptotic cell and was counted as one apoptotic cell. The rate of TUNEL-positive cells (apoptotic index) was calculated for each section by examining about 2,000 cancer cells, excluding the keratinized portion (cancer pearl) in the central part of the carcinoma nests in the well χ^2 and moderately differentiated SCC.

Statistical Analysis

The clinicopathological features and the histochemical results were analyzed by the χ^2 test or the Mann-Whitney test. The significance of correlation among the monocytic count, MVD, apoptotic index, and Ki-67 labeling index was evaluated by Pearson's analysis. Survival rates were analyzed by the Cox-Mantel test. Statistical significance was defined as $p < 0.05$.

RESULTS

Expression of MCP-1, CCR-2, and TP

In esophageal SCC, a positive reaction for MCP-1 was revealed in the cytoplasm of cancer cells and/or infiltrating stromal cells (Fig. 1A). Thirty-one SCC (55.4%) and 16 SCC (28.6%) were positive for MCP-1 in cancer and stromal cells, respectively. There was a significant correlation between MCP-1 expression in cancer and stromal cells. CCR-2 was expressed in infiltrating stromal cells in all SCC, and occasionally, in vascular endothelial cells in 15 SCC (26.8%).

TP was expressed in both cancer cells and stromal cells, which are considered to be mainly macrophages (Fig. 1B) because most of them showed CD68-positive staining. TP expression in the stromal cells was observed in 43 of 56 SCC (76.8%). There was a significant correlation between MCP-1 expression in cancer cells and TP expression in stromal cells (Table 1).

MCP-1 Expression and Monocytic Count/Microvessel Count/Ki-67 Labeling Index/Apoptotic Index

Monocytic count and MVD were calculated in the immunostained sections (Figs. 1C and D). Monocytic count in SCC with MCP-1 expression in cancer or stromal cells was higher than that without (Table 2). MVD in SCC with MCP-1 expression in cancer or stromal cells was higher than that without it.

Ki-67 labeling index was calculated in the sections that were immunostained (Fig. 1E). Ki-67 labeling index in SCC with MCP-1 expression in cancer cells was higher than that without, while there was no difference between SCC with and without MCP-1 expression in stromal cells (Table 2). The apoptotic index was calculated in the sections stained by TUNEL (Fig. 1F). The apoptotic index in SCC with MCP-1 expression in cancer cells was lower than that without, while there was no difference between SCC with and without MCP-1 expression in stromal cells (Table 2).

Correlations between monocytic count and MVD, Ki-67 labeling index, and apoptotic index are shown in Figure 2. There was a significant correlation between monocytic count and MVD, and there was an inverse correlation between monocytic count and apoptotic index.

Table 2. MCP-1 Expression and Histochemical Results in Esophageal SCC

Variable	MCP-1 Expression					
	Cancer Cells			Stromal Cells		
	Positive (n = 31)	Negative (n = 25)	*p* Value	Positive (n = 16)	Negative (n = 40)	*p* Value
Monocytic count	254.1 ± 84.3c	126.7 ± 73.5c	<0.0001	258.9 ± 92.9c	172.6 ± 95.1c	0.004
Microvessel density	33.0 ± 10.0e	19.5 ± 8.90	<0.0001	32.7 ± 10.1e	24.7 ± 11.5e	0.0175
Ki-67 labeling index	53.5 ± 7.5b	48.6 ± 10.0e	0.04	49.8 ± 7.20	51.8 ± 9.60	0.38
Apoptotic index	1.40 ± 0.6c	1.90 ± 0.63	0.0013	1.50 ± 0.65	1.70 ± 0.69	0.368

MCP-1, macrophage chemoattractant protein-1.

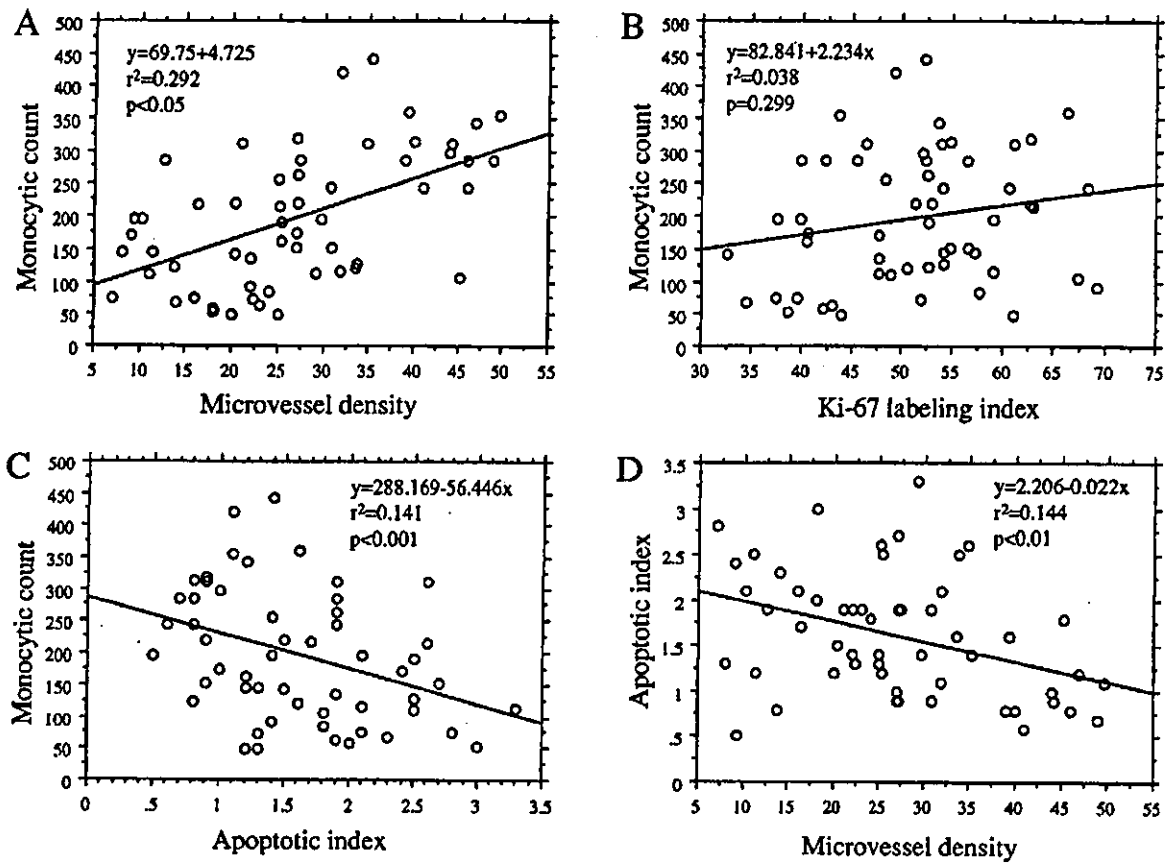

Figure 2. Relationships among monocytic count, microvessel density, Ki-67 labeling index, and apoptotic index. (*A*) Monocytic count and microvessel density. (*B*) Monocytic count and Ki-67 labeling index. (*C*) Monocytic count and apoptotic index. (*D*) Apoptotic index and microvessel density. There was a significant correlation between the monocytic count and the microvessel density, and there was an inverse correlation between the apoptotic index and the monocytic count, or the microvessel count.

Correlation with Clinicopathological Findings and Survival Rates

Regarding the relationship between MCP-1 expression and the clinicopathological features of the patients with esophageal cancer, MCP-1 expression in cancer cells was correlated with the depth of invasion, histological venous invasion, lymph node metastasis, and occurrence of clinical distant metastasis after surgery. However, there was no correlation between MCP-1 expression in stromal cells and the clinicopathological features (Table 3). The monocytic count in SCC with venous invasion, lymph node metastasis, and distant metastasis after surgery was higher than that without them, respectively (Table 4).

Five-year survival rate in esophageal SCC patients with MCP-1 expression was lower than that without (Fig. 3A). When the patients were divided into two groups based on the mean value of the monocytic count, the 5-yr survival rate in esophageal patients with a high monocytic count showed a worse outcome than that with a low monocytic count (Fig. 3B).

DISCUSSION

MCP-1 is a chemokine, which is thought to be a potent angiogenic factor. Ueno *et al.* (20) reported that the concentration of MCP-1 in breast cancer tissue was closely correlated with the concentration of TP and VEGF, which are major components of angiogenic factors. Furthermore, Saji *et al.* (21) reported a significant correlation between MCP-1 expression and neovascularization by histochemical evaluation in breast cancer. In the present study, MVD in the esophageal SCC with MCP-1 expression of cancer cells or stromal cells is higher than that without. Therefore, MCP-1 expression may play an important role in angiogenic promotion in esophageal SCC.

Infiltration of macrophages in tumor stroma is an important role for angiogenesis in several malignancies, including breast cancer (22) and oral SCC (23). In the present study, the monocytic count in esophageal SCC with MCP-1 expression was higher than that without it, and the monocytic count showed a significant correlation with MVD in esophageal

Table 3. MCP-1 Expression and the Clinicopathological Features in Esophageal SCC

Variable	No. of Patients	MCP-1 Expression: Cancer Cells Positive (n = 31)	Cancer Cells Negative (n = 25)	Cancer Cells p Value	Stromal Cells Positive (n = 16)	Stromal Cells Negative (n = 40)	Stromal Cells p Value
Sex							
Man	42	23	19	0.88	13	39	0.78
Woman	14	8	6		3	11	
Histological type of SCC							
Well- and moderately	47	25	22	0.36	15	32	0.2
Poorly	9	6	3		1	8	
Depth of invasion							
Submucosa	13	4	9	0.042	4	9	0.54
Muscularis propria or deeper	43	27	16		12	31	
Lymphatic invasion							
Positive	44	27	17	0.83	14	30	0.25
Negative	12	4	8		2	10	
Venous invasion							
Positive	31	23	8	0.0016	12	19	0.56
Negative	25	8	17		4	21	
Lymph node metastasis							
Positive	30	22	8	0.0037	8	22	0.73
Negative	26	9	17		8	18	
Distant metastasis after surgery							
Positive	16	14	2	0.002	7	9	0.11
Negative	40	17	23		9	31	

SCC. This suggests that macrophage recruitment might play an important role in tumor angiogenesis of esophageal SCC. Furthermore, these macrophages infiltrating in esophageal SCC may be identified as tumor-associated macrophages. On the other hand, TP, which acts as an angiogenic factor for tumors, is considered to be a protumor marker of infiltrating macrophages in several tumors (13). In the present study, TP was expressed in the infiltrating stromal cells that were identified mainly as macrophages, in esophageal SCC, and 76.7% of esophageal SCC had TP-expressed macrophages. MCP-1 expression was closely associated with TP expression in esophageal SCC, which suggests that MCP-1 produced by SCC cells stimulates infiltration of macrophages, which have CCR-2, in cancer stroma. Consequently, MCP-1 promotes tumor angiogenesis by angiogenic mediators, such as TP released from these infiltrating macrophages. Moreover, these macrophages may also play an important role in recruitment of macrophages because MCP-1 was expressed in infiltrating macrophages themselves, which indicates that there may be an autocrine function of MCP-1 on macrophages via the CCR-2. Interaction between cancer and stromal cells may be important for the tumor environment, including angiogenesis in esophageal SCC.

Table 4. Monocytic Count and the Clinicopathological Features

Variable	Monocytic Count	p Value
Histological type of SCC		
Well- and moderately	193.4 ± 101.4d	0.6
Poorly	217.2 ± 106.5d	
Depth of invasion		
Submucosa	1654.4 ± 96.8a	0.17
Muscularis propria or deeper	206.9 ± 102.1i	
Lymphatic invasion		
Positive	205.7 ± 102.2n	0.24
Negative	166.3 ± 97.0e	
Venous invasion		
Positive	233.0 ± 96.3e	0.003
Negative	152.9 ± 91.3e	
Lymph node metastasis		
Positive	224.1 ± 108.6m	0.035
Negative	166.2 ± 84.6e	
Distant metastasis after surgery		
Positive	290.1 ± 79.5e	<0.0001
Negative	160.1 ± 84.7e	

In addition, Salcedo *et al.* (24) reported that MCP-1 may also have a direct effect in tumor angiogenesis inducing chemotaxis of endothelial cells of blood vessels. Ohta *et al.* (25) reported that CCR-2 was immunohistochemically detected in monocytic cells but not in vascular endothelial cells. In the present study, CCR-2 was expressed in the endothelial cells of blood vessels in esophageal SCC. It was suggested that MCP-1 may be a potent angiogenic promoter by migration of the endothelial cells, although it was not a major promotion.

In the esophageal SCC patients with distant metastases after surgery, high monocytic counts were shown in the present study. These infiltrating monocytes may act as the tumor-associated macrophages, mentioned above. It suggested that these macrophages may play important roles for not only angiogenesis to maintain and control tumor environment but distant metastases of tumor cells in esophageal SCC, because

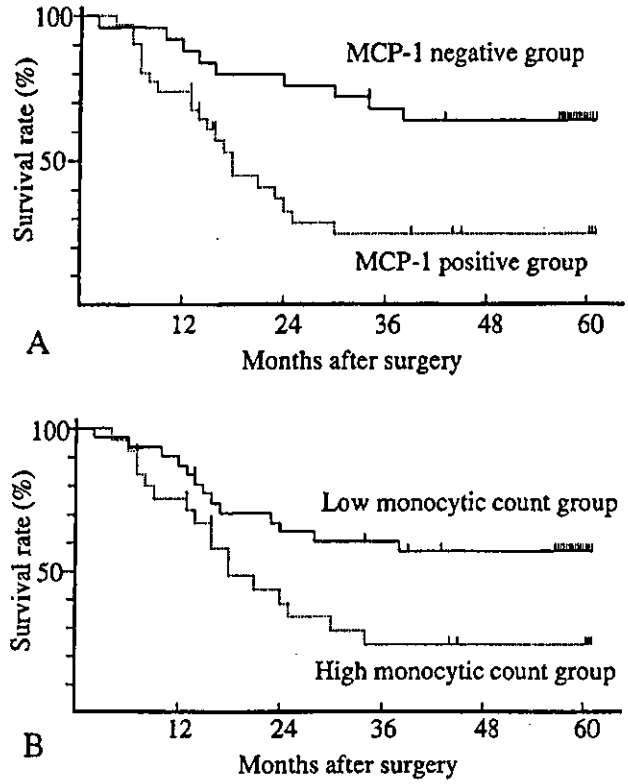

Figure 3. Survival curves in esophageal cancer patients. (*A*) Five-year survival rate in patients with MCP-1 expression was worse than that without it (24.5% *vs* 63.8%; $p < 0.01$). (*B*) Regarding monocytic count, the patients were divided into two groups: a high and a low monocytic count group. Five-year survival rate in patients with a high monocytic count was worse than those with a low monocytic count (24.2% *vs* 56.9%; $p<0.05$).

these macrophages may supply tumors many growth and angiogenic factors, including TP and cytokines. Previously, in esophageal SCC patients with TP expression (14) and the other growth factors (15), distant metastases has been frequently shown.

Regarding proliferative activity of esophageal SCC, we investigated the findings of Ki-67 labeling index and apoptotic index. Ki-67 labeling index of esophageal SCC with MCP-1 expression in cancer cells was higher than that without, while there was no difference in the Ki-67 labeling index between esophageal SCC with and without MCP-1 expression in stromal cells. Furthermore, the apoptotic index of esophageal SCC with MCP-1 expression in cancer cells was lower than that without, while there was no difference in the apoptotic index between esophageal SCC with and without MCP-1 expression in stromal cells. Tumor angiogenesis may be closely associated with apoptosis of tumor cells (26). In the present study, MVD is inversely correlated with the apoptotic index, and MVD was correlated with the monocytic count in esophageal SCC. Therefore, MCP-1 expression of cancer cells may contribute to macrophage infiltration and angiogenesis, probably as protumor macrophages, and thus MCP-1 expression may be interactively associated with proliferative activity and apoptosis in esophageal SCC.

Regarding the clinicopathological features of patients with esophageal SCC, MCP-1 expression of cancer cells and macrophage infiltration were associated with histologic venous invasion and distant metastasis after surgery. As a result, 5-yr survival rate was worse in patients with MCP-1 expression or with a high monocytic count. Leek *et al.* (11) reported that macrophage infiltration was associated with angiogenesis and a poor prognosis in patients with invasive breast cancer. Therefore, in esophageal SCC, MCP-1 expression and macrophage infiltration also play important roles in not only angiogenic promotion but clinicopathological aggressive behavior as well, and they may be useful for their ability to predict the clinical outcome of esophagectomized patients.

In conclusion, MCP-1 expression and macrophage infiltration may be associated with angiogenic promotion. MCP-1 expression may be interactively associated with macrophage infiltration; MCP-1 may play an important role in tumor angiogenesis through production of angiogenic factors, such as TP, by recruited macrophages in esophageal SCC. Furthermore, CCR-2 expression in vascular endothelial cells may participate partially in angiogenesis. Clinicopathologically, patients of esophageal SCC with MCP-1 expression have no favorable prognosis.

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DISEASES OF THE ESOPHAGUS

Case report

Thoracoscopic enucleation of esophageal stromal tumor

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SUMMARY. **Gastrointestinal stromal tumor is a rare entity, especially in the esophagus. We report a patient with a stromal tumor of the esophagus who underwent a thoracoscopic enucleation of the tumor. The patient was a 61-year-old man complaining of slight dysphagia. A submucosal tumor of the middle thoracic esophagus was found endoscopically. The tumor was approximately 4.0 cm in diameter measured by endoscopic ultrasonography. On 17 May 2001, thoracoscopic enucleation of the esophageal tumor was performed using a Kodama Di-suction. The Kodama Di-suction was useful for the thoracoscopic enucleation of the submucosal tumor of the esophagus, acting as both a dissector and a sucker. The patient's course was uneventful after surgery. Histopathologically the esophageal tumor revealed a high cellularity, consisting of spindle cells, and the tumor cells were immunohistochemically positive for CD34 and c-kit protein, but not for a-smooth muscle actin or S-100 protein. From these findings, the esophageal submucosal tumor was diagnosed as gastrointestinal stromal tumor, distinguished from leiomyoma.**

KEY WORDS: **esophagus, GIST, Kodama Di-suction, thoracoscopy.**

INTRODUCTION

Gastrointestinal stromal tumor (GIST) is the most common mesenchymal tumor of the stomach and the intestine, however, in the esophagus, leiomyoma is most common.[1] Herein, we report a patient with a GIST of the esophagus which was enucleated thoracoscopically, using a thoracoscopic instrument functioning as a sucker as well as a dissector.

CASE REPORT

A 61-year-old man, complaining of slight dysphagia, was monitored endoscopically for an esophageal submucosal tumor from July 2000, and admitted to Shinshu University Hospital to remove this tumor surgically on 7 May 2001. A barium meal study showed a tumor with a smooth surface in the right wall of the middle thoracic esophagus, and approximately 4 cm in longitudinal diameter (Fig. 1). Endoscopy showed a protruded lesion covered with a normal mucosa in the middle thoracic esophagus (Fig. 2A), and no morphological change of the tumor was detected in endoscopic findings between July 2000 and March 2001. Biopsy specimens taken from the tumor in July 2000 histologically revealed a stromal tumor. An endoscopic ultrasonogram (EUS) in July 2000 showed that the esophageal tumor was 3.5 × 2.0 cm in diameter, and originated from the proper muscle layer of the esophagus (Fig. 2B). However, in EUS in May 2001, the diameter of the tumor was 4 × 2.5 cm in diameter. A computed tomogram showed the esophageal tumor with heterogeneous density, and no metastasis in the lung and the liver.

On 17 May 2001, thoracoscopic enucleation of the submucosal tumor of the esophagus was performed under general anesthesia administered using a double-lumen endothoracheal tube. A 10-mm-video-trocar was inserted into the 7th intracostal space on the mid-axillary line of the right lateral chest. Four 10 mm-trocars were inserted into the intracostal spaces on the anterior or posterior axillary lines for operative and assisting instruments. As the lung was collapsed, the tumor was found in the middle thoracic esophagus covered with the

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Fig. 1 A protruding tumor with a smooth surface is detected in the middle thoracic esophagus.

mediastinal pleura (Fig. 3A). The pleura was incised over the lesion, the esophageal longitudinal muscle was incised at the midpoint of the tumor, and the white tumor was exposed (Fig. 3B). The tumor was then enucleated by careful dissection using a Kodama Di-suction (Sumitomo Bakelite Co., Ltd, Nagoya, Japan; Fig. 3C). A few blood vessels fed the tumor, and they were cut using a coagulation shear. Following removal of the tumor, an endoscope was orally inserted into the esophagus and no injury to the esophageal mucosa was detected. In addition, no air-bubble infused into the esophagus from the endoscopy was detected in the thorax. The esophageal muscle layer was closed longitudinally using absorbal sutures. The tumor was inserted into a bag and extracted through the port site slightly expanded to 2.5 cm (Fig. 3D). The patient's course was uneventful after surgery.

Macroscopically, the enucleated tumor was white, $4.5 \times 3.3 \times 2.5$ cm in size, and showed neither bleeding nor necrotic tissue. Histopathologically, dense, elongated spindle-shaped tumor cells with a dense chromatin nucleus and basophilic cytoplasm were observed (Fig. 4A). The mitotic figures were hardly observed in the tumor cells. In immunohistochemistry, almost all the tumor cells were positive for CD34 and CD117 (c-kit protein) antigens while these cells were negative for S-100 protein and a-smooth muscle actin (Fig. 4B–E). From these findings, the tumor was diagnosed as GIST of the esophagus. The Ki-67 positive ratio of the tumor nuclei (labeling index) was about 0.5% (Fig. 4F).

DISCUSSION

GIST is a rare entity of the esophagus. Previously, many patients with a leiomyoma of the esophagus have been reported,[2,3] however, esophageal stromal tumor with CD34 and CD117 coexpression, which is considered to be GIST, is rare.[1] Recently, the concept of GIST has been accepted histopathologically and clinicopathologically in mesenchymal tumors, and

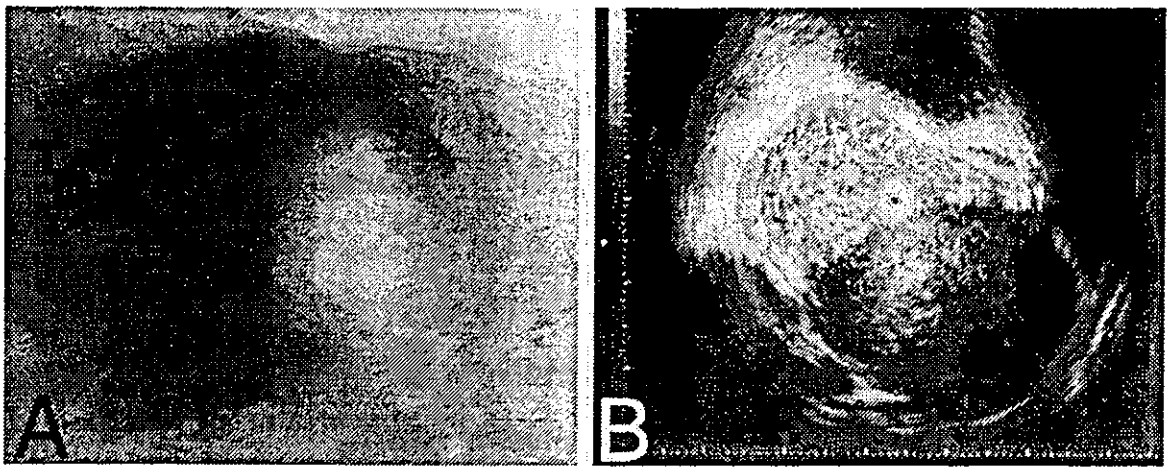

Fig. 2 Endoscopic examination: (A) esophagoscopy shows a submucosal lesion covered with normal esophageal mucosa; (B) endoscopic ultrasonogram shows a submucosal tumor continuing with the proper muscle layer of the esophagus.

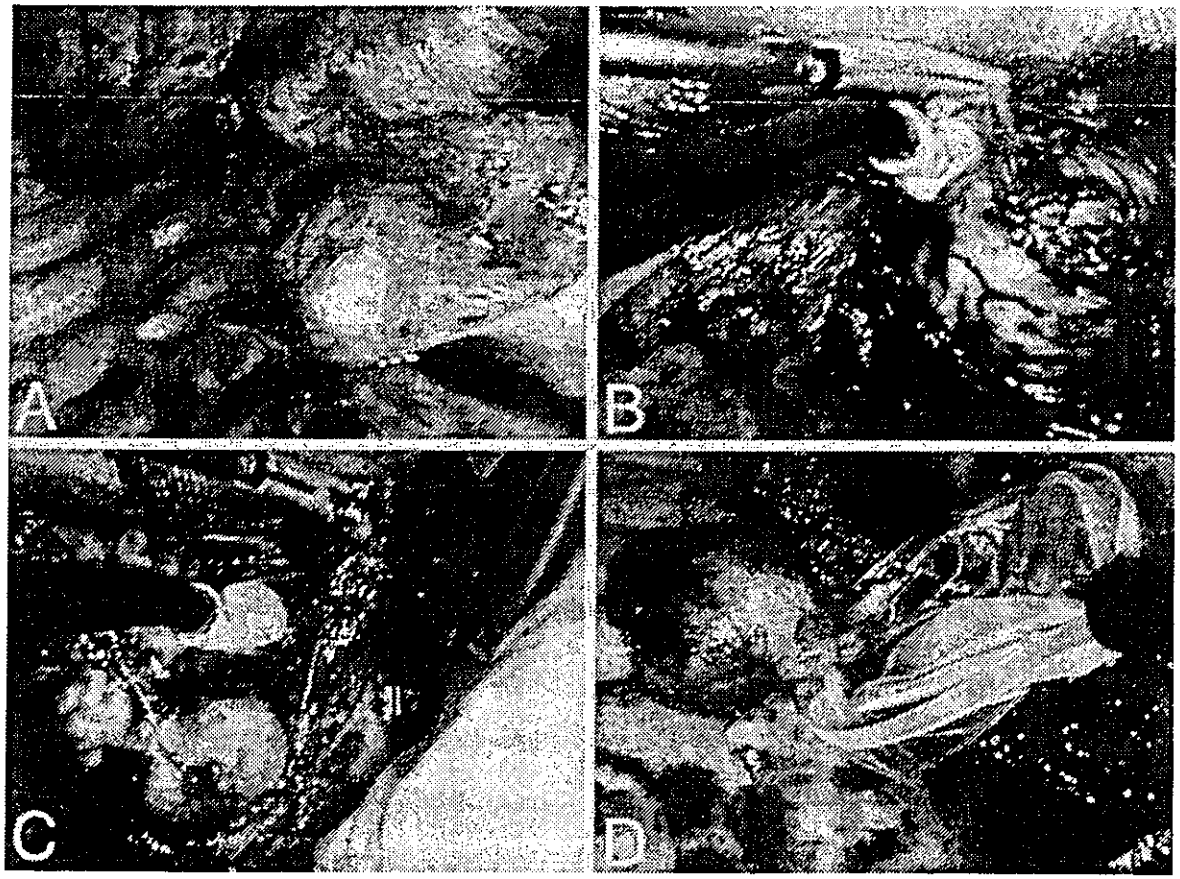

Fig. 3 Thoracoscopic findings: (A) the esophageal tumor projects into the right thoracic space; (B) the white submucosal tumor is exposed in the proper muscle layer of the esophagus; (C) the tumor is enucleated with a Kodama Di-suction (a useful instrument for dissection of the tumor and as a sucker); (D) the enucleated tumor is collected in a bag.

GIST is considered separate from myogenic or neurogenic mesenchymal tumor of the GI tract.[4,5] Miettinen *et al.* first documented and analyzed GIST of the esophagus, and histologically distinguished them from leiomyomas and leiomyosarcomas.[1] In the present case, the lesion was endoscopically a submucosal tumor of the esophagus, and judged to have originated from the proper muscle layer by EUS before surgery. Histopathologically, the removed esophageal tumor revealed a high cellularity of spindle cells with neither reaction for a smooth muscle actin nor S-100 protein but with positive reactions for both CD34 and CD117. These clinicopathologic findings showed that this esophageal submucosal tumor was a GIST.

GIST has a malignant potential.[6] Patients with a large GIST are associated with a poor prognosis, and surgical treatment for these patients is controversial.[7] Laparoscopic surgery has been widely available for patients with a small GIST, and these patients show a good prognosis. In esophageal stromal tumor, Miettinen *et al.* reported that nine of 17 patients with esophageal GIST died of the disease,[1] although surgical treatment was not analyzed and discussed in these patients. All patients who died of the disease had a large GIST between 5 cm and 25 cm in size. In esophageal GIST, tumor size may be important for surgical management and patient prognosis. In the present case, because the tumor enlarged over 11 months on EUS and the patient had dysphagia, the tumor was enucleated. Fortunately, the enucleated GIST was 4.5 cm in diameter, and showed histopathologically low mitosis and low Ki-67 labeling index.

For esophageal leiomyomas, several surgical or endoscopic approaches have been reported. The traditional method using thoracotomy was associated with high surgical stress and invasiveness. Several thoracoscopic procedures have recently been applied for benign esophageal disorders including esophageal leiomyomas.[8,9] For surgical treatment of esophageal submucosal tumors, the thoracoscopic approach showed many benefits including minimal scarring, decreased pain and rapid recovery after surgery.[8-10] The management and technique of thoracoscopic surgery in this patient with GIST was similar to those of previous studies of esophageal leiomyoma. In this situation, perforation of the

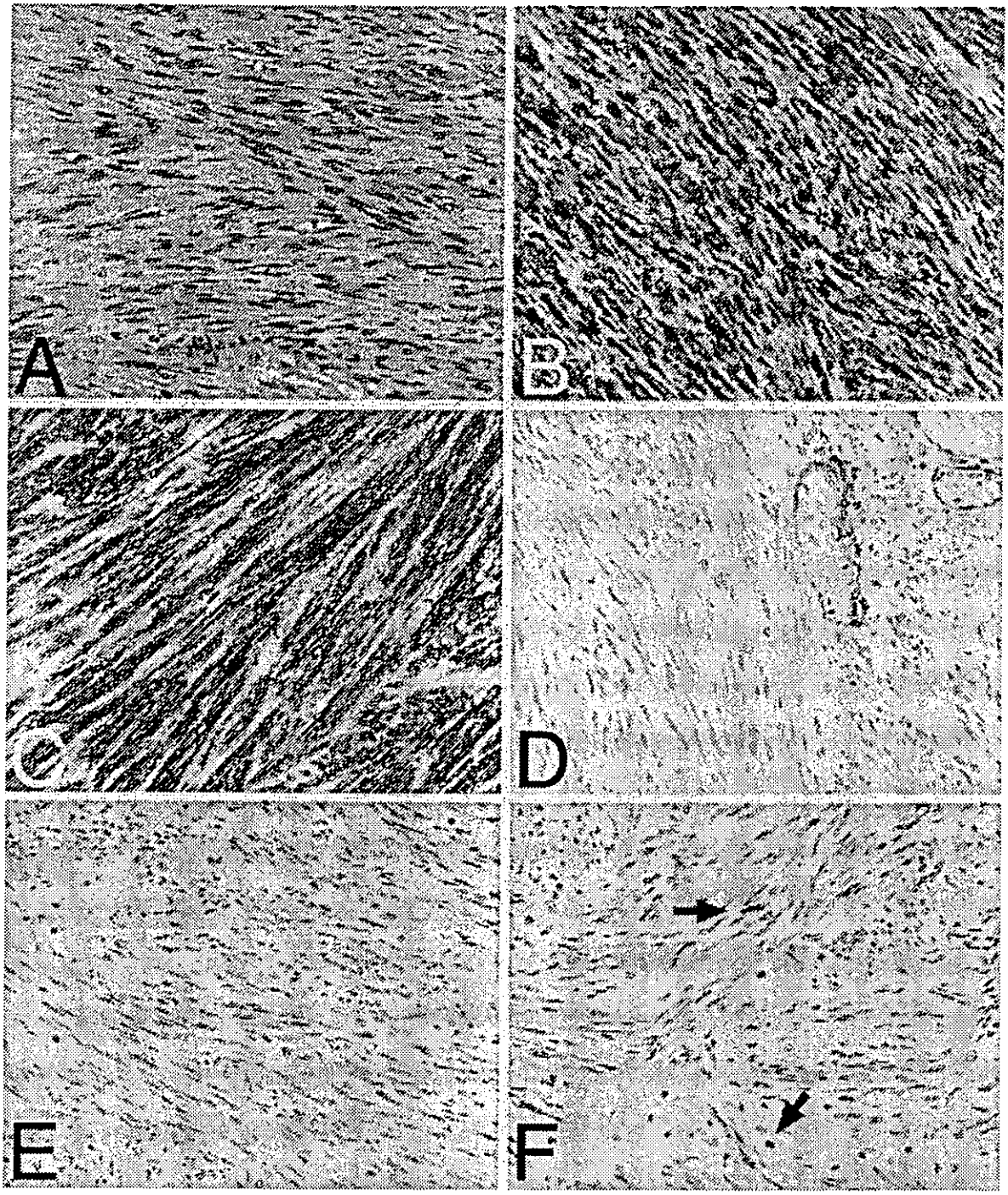

Fig. 4 Histopathologic findings: (A) HE staining; (B) CD 34 immunostaining; (C) CD 117 immunostaining; (D) α-smooth muscle actin immunostaining; (E) S-100 protein immunostaining; (F) Ki-67 immunostaining. The spindle-shaped tumor cells showed a positive reaction for CD 34 and CD 117 antigens, but no reaction for α-smooth muscle actin or S-100 protein. Ki-67 immunostaining produced a positive in the nuclei of the tumor cells (arrowed).

esophagus may be the most common complication developing from dangerous complications such as leakage and abscesses in the mediastinum and the thorax.[9] In previous studies of esophageal leiomyoma, an enucleation of the tumor was performed using a mounted swab or a coagulation shear. In the present case, we used the Kodama Di-suction thoracoscopically for the removal and enucleation of the esophageal stromal tumor.[11] Although this GIST was fed by several blood vessels and these vessels were cut using the coagulation shear, the Kodama Di-suction, which functions as both a

dissector and a sucker, may be useful for the enucleation of submucosal tumors because there was no injury of the esophageal mucosa in the present case.

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第7回肝移植臨床検討会

生体肝移植後の再発C型肝炎の臨床病理学的検討

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Clinicopathologic analysis of recurrent hepatitis C after living donor liver transplantation

key words：再発C型肝炎，生体肝移植，拒絶反応，抗ウイルス療法

肝移植術後においてC型肝炎ウイルス(HCV)の出現はほぼ必発であり，移植後患者の70～80%において肝機能異常を伴う再発C型肝炎を発症するとされる[1]．

当初，再発C型肝炎は，再発B型肝炎に比し予後が良好であるといわれてきた．しかし最近，術後5年で再発例のうち20～30%が肝硬変となり，さらに術後10年で50%が肝硬変となると報告されており，肝移植後の免疫抑制とそれによるHCVの著明な増殖がその原因と考えられている[2]．また，生体肝移植後の再発C型肝炎は，脳死全肝移植後に比し重症化するとの報告も散見され，これら移植後のC型肝炎の予後解析が急務となっている[3]．

今回，信州大学移植外科にて生体肝移植が施行されたC型肝炎症例の術後の再発肝炎を中心に，臨床病理学的に検討したので報告する．

対象と方法

1990年6月～2004年3月まで，当施設にて18例のC型肝炎・肝硬変症例に対して生体肝移植が施行された．これら18例の移植前の臨床・検査成績は，表1のとおりである．

18例全例で術後定期的にHCV-RNAが定量測定され，再発C型肝炎の診断は組織学的あるいは臨床的に行われた．臨床的再発は，生検は施行されないものの肝機能異常とHCV-RNA陽性所見が認められ，かつ他の肝機能異常の原因が臨床的に否定されたものとした．また，再発後複数回の組織学的検査が施行された症例において，組織所見の進行について検討した．再発と診断され，抗ウイルス療法が可能な状態と判断された症例でインターフェロンα-2b(300万単位，週3回投与)とリバビリン(600～800 mg/日，内服)による治療を施行した．

肝生検が施行された再発症例を，臨床的に急性拒絶反応が先行しステロイドパルス療法が施行され，その後に再発と診断された症例とそうでない症例とにわけ，臨床・病理学的に比較検討した．

C型肝炎症例18例の生体肝移植後生存率については，Kaplan-Meier法により検討した．また，患者生存に関与すると考えられる因子について考察した．

表1 C型肝炎症例18例の移植前所見

臨床・検査所見	18
1. 性別：male/female	14/4
2. 平均年齢(齢)	55(32～69)
3. HCC合併例	12(67%)
4. HCVジェノタイプ1b	18(100%)
5. 移植グラフト：left lobe/others	17/1
6. 平均GV/SV(%)	37(34～49)
7. 免疫抑制：FK/CsA	16/2

表2 再発C型肝炎の組織所見

初回再発診断時(8例)
- F0/A1-A2

肝生検による長期フォローアップ例(4例)
- F0/A1(POD 40) ⟹ F2/A2 (POD420)
- F0/A1(POD 52) ⟹ F1/A1(POD211)
- F0/A2(POD142) ⟹ F2/A1(POD1201)
- F0/A1(POD 50) ⟹ F3/A2(FCH?)(POD169)

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表3　急性拒絶反応→再発C型肝炎症例

	Case 1	Case 2	Case 3
Age(yr)	62	61	60
Timing ACR	POD 25	POD 126	POD 43
Timing hepatitis	POD 37	POD 142	POD 50
AST max(U/L)	278(POD37)	607(POD133)	263(POD157)
T.Bil max(mg/dL)	4.5(POD72)	26.6(POD146)	55.2(POD183)
IFN + RIBA	POD40	POD145	(−)
Prognosis	46 mo. Alive	47 mo. Alive	6 mo. Dead. FCH?

表4　急性拒絶反応→再発C型肝炎症例の組織所見

Case	Biopsy No.	POD	Eosinophils	Bile duct damage	Endotheliitis	Kupfer cell hypertrophy	Focal cell loss	Apoptosis count	Diagnosis
1.	6505	25	++	+	++		+	1.3	ACR
	6560	29	+		+	+		1.7	ACR
	6743	37		+			+	5.3	Hepatitis
2.	1544	126		++	++	+	+	0.6	ACR
	1852	133			+	+	+	1.3	ACR
	3885	142	+			+		0.3	Hepatitis
3.	1000	43		+	+	+	+	3.3	ACR
	1134	50		+		+	+	4.7	Hepatitis
	1270	57		+	+		+	2.1	ACR
	1540	75				+	+	2.5	Hepatitis

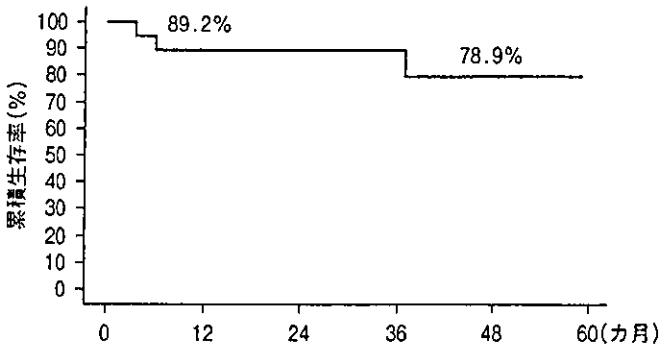

図1　C型肝炎症例の移植後生存率(18例)

結　果

移植後，18例全例で血清HCV-RNAは陽性化した．うち10例でHCV-RNAはアンプリコアモニター法にて定量測定され，その最高値は320～850以上KIU/mLであり，8例ではその定量のためにリアルタイムPCRが用いられ，その術後最高測定値は3.7×10^7～7.0×10^9copies/mLであった．

18例中10例(56%)でC型肝炎が再発した．8例の再発肝炎診断は術後89日(中央値31～132日)に組織学的に行われた．8例の再発初回診断時の組織学的所見はF0/A1-A2であり，うち4例でフォローアップの肝生検が施行され，これら4例はいずれの症例でも線維化の進行が認められた(表2)．

肝炎再発症例中6例(60%)でインターフェロン＋リバビリンによる抗ウイルス療法が導入された．しかし，2例でインターフェロンによる精神症状が出現し，また，拒絶反応と感染症によりそれぞれ1例でこれら治療が中止された．最終的には2例でのみ6カ月以上の長期投与が可能であったが，この2例においてもHCVは排除されず，肝機能の改善が認められたのみであった．

臨床的に拒絶反応が先行しない再発肝炎症例5例では，一過性のトランスアミナーゼ値の上昇が特徴的であり，診断後1カ月以内に抗ウイルス療法の施行にかかわらずトランスアミナーゼ値は正

表5 C型肝炎術後死亡症例

	症例1	症例2	症例3
年齢(歳)	69	64	60
HCC	(+)単発	(+)16個	(−)
術前MELD	21	9	15
術後合併症	胆管狭窄		拒絶反応
術後(カ月)	3	25	6
死因	アスペルギルス，肝不全	HCC再発(肺・脳)	アスペルギルス，FCH？

常化した．組織学上，異常所見が肝小葉内に限局するlobular hepatitisの像を呈し，C型肝炎(再発)の診断は容易であった．

一方，拒絶反応が先行し，その治療後再発C型肝炎と診断された3例では，トランスアミナーゼ，ビリルビンのピーク値が高値であり，2例ではトランスアミナーゼ値の正常化に5カ月以上を要し，1例は術後約6カ月に肝不全で死亡した．死亡症例の死亡直前の組織所見は，fibrosing cholestatic hepatitis(FCH)に類似した像を呈していた(表3)．

これら3例の初回3～4回の具体的肝生検所見を検討した(表4)．eosinophiliaとendotheliitisは，再発肝炎に比し拒絶反応に有意に高頻度に認められたが，胆管障害と肝小葉内の変化は再発肝炎と拒絶反応ともに認められた．しかし，小葉内のapoptotic bodyの数は，再発肝炎で高値の傾向があった．

C型肝炎症例18例の移植後フォローアップ期間は34カ月(中央値3.5～60カ月)であり，1，2年患者生存率は89.2%，5年患者生存率は78.9%であった．再移植が施行された症例はなく，グラフト生存率も同値であった(図1)．死亡例はそれぞれ，1例では高齢＋MELDスコア高値のリスクファクターを有し，1例では高度進行肝細胞がんを術前合併し，1例では移植後の難治性拒絶反応治療による重症感染症，重症再発C型肝炎が死因となった(表5)．

考 察

C型肝炎に対する肝移植の予後に影響する主な因子として，再発C型肝炎と再発肝炎以外の移植時年齢，術前状態などのレシピエント因子があげられる[2,4]．再発C型肝炎の進行の防止・発症予防には，HCVそのものに対する抗ウイルス療法が必須であるが，実際には今回の検討で示されたように，インターフェロンとリバビリンにはさまざまな副作用があり，このような副作用により治療導入後の中止・脱落が高頻度である[5]．

また，ペグインターフェロンとリバビリンによる治療にても，いまだ満足な治療成績が得られていないのが実情であり，このような状況下で，肝移植後に高ウイルス血症が認められるものの，肝機能が安定している際には抗ウイルス療法を導入しない施設も多い．今後は，より有効な治療法の開発，治療の開始時期，投与量，投与期間など，さらなる検討が望まれる．

拒絶反応の治療後に再発C型肝炎と診断された症例では，2例で肝機能回復に時間を要し，1例は肝不全へ進行した．これらの所見は，拒絶反応に対するステロイドパルス療法，OKT3投与は肝細胞内のHCV増殖を促進するため，重症・進行性の再発C型肝炎発症に寄与するという報告を支持するものと考えられる．

C型肝炎症例の肝移植後においては，拒絶反応，それに対する治療がその予後に影響を及ぼす可能性を常に留意すべきである．場合によりC型肝炎症例では，組織検査上，軽微な拒絶反応と診断される症例では，無治療のまま経過を観察するなどの非積極的な拒絶反応の治療も必要であるかもしれない．今回の検討では，拒絶反応と再発C型肝炎の組織学的所見のオーバーラップが明らかとなったが，不必要な免疫抑制強化を避けるためにも，正確な拒絶反応の組織診断，拒絶反応と

再発肝炎の鑑別はきわめて重要である.

死亡例2例では，1例は69歳と高齢かつ術前のMELDスコアが高値であり，1例では高度進行肝細胞がんを合併し，移植後再発肝細胞がんが直接の死因であった．一般に生体肝移植においては，脳死肝移植に比し移植の適応は拡大されているものと考えられるが，これら2例の術後予後は移植適応決定について示唆を与えるものと考えられる.

C型肝炎症例の生体肝移植後5年生存率は比較的良好であった．18例中移植後5年以内での再発C型肝炎による死亡は1例のみであり，再発肝炎の短期予後に及ぼす影響は明らかでなかった．しかし，近年，再発C型肝炎が予後に影響する時期は移植後5～10年とされており，さらに正確な予後の判定には長期の経過観察が必要であると考えられる.

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