Prognostic significance of EpCAM expression in human esophageal cancer

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Abstract. EpCAM is a 40-kDa epithelial transmembrane glycoprotein that has a well-known role in Ca^{2+}-independent homophilic cell-cell adhesion. Recently, correlations between EpCAM expression and clinicopathological features have been investigated in various cancers. We examined EpCAM expression in surgical specimens from esophageal cancer patients (n=138), using real-time RT-PCR, immunohistochemistry and ELISA. The mean expression level of EpCAM mRNA in tumor tissues was significantly higher than that in corresponding normal tissues (P<0.0001). Immunohistochemically, positive staining for EpCAM was found in 135 (97.8%) of the 138 primary tumor specimens. EpCAM expression was correlated with tumor depth (P=0.0005), stage (P=0.0037), blood-vessel invasion (P=0.0397) and infiltrative growth pattern (P=0.0015). The survival rates of patients with tumors with high EpCAM expression was significantly higher than those for patients with tumors with low EpCAM expression (P=0.0213). Furthermore, the serum EpCAM levels of patients with esophageal cancer were significantly higher than those of normal volunteers (P=0.0221). The survival rates of patients with a high EpCAM level in the peripheral vein were also significantly higher than those for patients with a low serum EpCAM level (P=0.0291). The serum EpCAM level in the peripheral vein was independently associated with prognosis (P=0.0074; hazard ratio 7.40). Tumor-specific EpCAM expression and release into the circulation may serve as effective immunotherapy in esophageal cancer patients.

Introduction

EpCAM (also referred to as 17-1A, ESA and EGP40) is a 40-kDa epithelial transmembrane glycoprotein encoded by the GA733-2 gene, which is located on the long arm of chromosome 4 (1,2). The EpCAM protein consists of two epidermal growth factor-like extracellular domains, a cysteine-poor region, a transmembrane domain, and a short cytoplasmic tail. The name EpCAM was first suggested by Litvinov *et al*, to more precisely reflect the function and tissue specificity of the protein (3,4).

EpCAM has a well-known role in Ca^{2+}-independent homophilic cell-cell adhesion,(4) and is also known to be involved in the signaling cascade related to proliferation, differentiation and apoptosis, in addition to other adhesion molecules. In tumor malignancy, EpCAM can be thought of as having a dual role (5). Hence, EpCAM induces dissociation of cadherin-mediated adhesion to promote invasion and metastasis (6-8), but also plays a role as an adhesion molecule that suppresses metastasis by holding cells in place (9). Until recently, the regulation of the EpCAM gene has been unclear, although the promoter region that regulates EpCAM transcription has been cloned and shown to be negatively regulated by TNF-α (10).

EpCAM is strongly expressed in cancers of various origins, including colon and rectum (11), prostate (12,13), liver (14,15), esophagus (16,17), lung (18,19), head and neck (20), and breast (21). Went *et al* used immunohistochemistry and tissue microarray analysis to detect EpCAM protein expression in various human cancers, and provided a review of EpCAM expression in cancers (22). Furthermore, correlations between EpCAM expression and clinicopathological features have been investigated in various cancers, including breast (23), lung (19), ovary (24), gallbladder (25), kidney (26), and stomach (27). The EpCAM expression level has been shown to be a predictor of survival in cancer patients, but the published results are inconsistent in this respect, and it is possible that EpCAM expression has a bidirectional effect regarding clinicopathological features and prognosis in cancer patients.

EpCAM has become one of the major targets for immunotherapy with monoclonal antibodies because of its specificity in cancerous lesions. For example, postoperative administration of EpCAM-specific murine monoclonal antibody (17-1A) in Dukes' C colorectal cancer patients led to prevention of distant metastasis and to prolonged survival after a 7-year follow-up period (28). Bispecific antibodies against EpCAM and CD3 (29,30) or B7 (31) have been engineered to improve

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cytotoxicity through synergistic effects of antibodies and T-lymphocytes.

We have investigated EpCAM expression in esophageal cancer patients using real-time reverse transcription (RT)-PCR and immunohistochemistry, and determined serum EpCAM levels in these patients using ELISA. Correlations of these data with clinicopathological features of the tumor and prognosis are explored, and we discuss the prospects for EpCAM-associated immunotherapy in esophageal cancer patients.

Materials and methods

Patients and samples. The study was performed on 138 patients with esophageal cancer who underwent potentially curative surgery without preoperative therapy at the Department of General Surgical Science, Gunma University Graduate School of Medicine, between 1983 and 2004. Tumor stage was classified according to the 6th edition of the tumor-node-metastasis classification of the International Union Against Cancer (UICC) (32). All patients signed informed consent forms according to our institutional guidelines. Information on gender, age, stage of disease, and histopathologic factors was extracted from medical records.

For preparation of fresh-frozen sections, normal and tumor tissues were collected separately from fresh specimens resected in the operating room. The tissue samples were put into tubes, frozen in liquid nitrogen and stored at -80˚C until use. Frozen tumor tissues and corresponding normal tissues from 49 patients were obtained for real-time RT-PCR analysis.

Prior to analysis, the resected specimens were fixed with 10% formaldehyde, embedded in paraffin blocks, cut into 4-μm thick sections, and mounted on glass slides. Paraffin-embedded sections were acquired from all 138 patients (121 males and 17 females). The median age of these patients was 62 years, with a range of 40-79 years, and the median survival time was 34.7 months, with a range of 1-211 months.

For ELISA, preoperative serum samples were acquired from 60 patients (54 males and 6 females). The median age of these patients was 62 years, with a range of 41-80 years, and the median survival time was 36.9 months, with a range of 4-68 months. Serum samples from 20 healthy volunteers without any cancer (average age 65.4 years) were assayed as normal controls.

Before surgery, venous blood was obtained from each patient. Blood samples were stored at 4˚C after collection, and the serum was separated from the blood by centrifugation at 1500 x g for 5 min. These samples were kept frozen at -80˚C without any incident of thawing until they were assayed.

RNA isolation and cDNA synthesis. Total RNA was extracted from fresh-frozen sections using an RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The quantity of isolated RNA was measured using an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Template cDNA was synthesized from 13.5 μg of total RNA with an Omniscript Reverse Transcriptase kit (Qiagen), random primer (hexadeoxyribonucleotide mixture) (Takara, Shiga, Japan) and ribonuclease inhibitor (Porcine liver, Takara). Total RNA was reverse-transcribed with 4 units of Omniscript reverse transcriptase in a reaction volume of 20 μl (60 min at 37˚C, 5 min at 93˚C, and finally on ice). The resultant cDNA samples were stored at -30˚C until analysis.

Real-time RT-PCR. Real-time RT-PCR analyses were performed on an ABI Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA) The standard reaction volume was 20 μl and contained 1X SYBR Green PCR Master Mix (ABI), 2.0 μl of cDNA template, and 1.0 μM of both forward and reverse primers. The initial PCR denaturation step was performed for 5 min at 95˚C, followed by 40 cycles of 60 sec at 95˚C (melting) and 60 sec at 60˚C (annealing/elongation). All reactions were performed in duplicate. The data were normalized to an internal control gene, ß-actin, to control for the amount of RNA in the preparation. Primers for EpCAM and ß-actin were used with reference to previously published assays (33,34). The primer sequences were as follows: EpCAM, 5'-CGCAGCTCAGGAAGAATGTG-3' (forward) and 5'-TGAAGTACACTGGCATTGACG-3' (reverse); ß-actin, 5'-CTCCTCCTGAGCGCAAGTACTC-3' (forward) and 5'-TCCTGCTTGCTGATCCACATC-3' (reverse).

Immunohistochemical staining. Immunohistochemical staining of the sections for EpCAM expression was performed by the standard streptavidin-biotin peroxidase complex (S-ABC) method, as described previously (35,36). Each 4-μm thick section was deparaffinized, rehydrated and incubated with fresh 0.3% hydrogen peroxide in methanol for 30 min at room temperature to block endogenous peroxidase activity. After rehydration through a graded series of ethanol treatments, the sections were autoclaved in 10 mM citrate buffer (pH 6.0) at 120˚C for 3 min, and then cooled to 30˚C. After rinsing in 0.1 M phosphate-buffered saline (PBS; pH 7.4), non-specific binding sites were blocked by incubation with 10% normal rabbit serum for 30 min. Sections were incubated with the murine monoclonal antibody KSA (NovoCastra, Medac GmbH, Hamburg, Germany) at a dilution of 1:50 in PBS containing 1% bovine serum albumin at 4˚C overnight. The sections were washed in PBS, incubated with biotinylated anti-murine IgG for 30 min at room temperature, and finally incubated in streptoavidin-biotin peroxidase complex solution (Nichirei Co., Tokyo, Japan). The chromogen, 3,3'-diamino-benzidine tetrahydrochloride, was applied as a 0.02% solution containing 0.005% H_2O_2 in 50 mM ammonium acetate-citrate acid buffer (pH 6.0). The sections were lightly counterstained in Mayer's hematoxylin and mounted. Negative controls were established by replacing the primary antibody with normal rabbit serum, and no detectable staining was evident in these controls.

EpCAM protein expression scoring. EpCAM overexpression was evaluated by calculating the total immunostaining score as the product of the intensity score and the staining rate. The intensity score was based on the estimated staining intensity (0, no staining; 1, weak; 2, moderate; 3, strong), and the staining rate was defined as the percentage of tumor cells with a particular intensity score. Each intensity score (IS: 0,1,2,3) was multiplied by its associated staining rate [SR (%)],

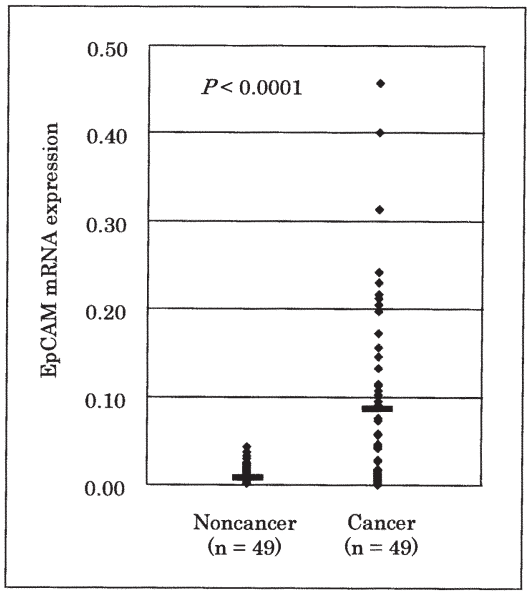

Figure 1. EpCAM mRNA expression in cancerous and non-cancerous esophageal tissues. Horizontal lines indicate means. The cancer tissues showed significantly higher EpCAM mRNA expression levels compared to non-cancerous tissues (P<0.0001, Wilcoxon signed-rank test).

and the total immunostaining score was calculated by summing the results over all staining intensities:

$$\text{total score} = \sum_{n=0}^{3} (\text{IS n x SR n}/100)$$

Serum EpCAM quantification. Before running the assay, all samples were thawed at 4˚C overnight and then diluted 1:3 using dilution buffer, based on the instructions in the kit protocol. For quantitative measurements of serum EpCAM levels, a double monoclonal sandwich enzyme immunoassay kit (Human EpCAM ELISA kit, BioVendor Laboratory Medicine, Inc.) was used. All samples were assayed in duplicate in a blinded fashion, and the mean was used for data analysis.

Statistical analysis. Statistical analysis was performed using the Wilcoxon signed-rank test, χ^2 test, Mann-Whitney U test, and Kruskal-Wallis test. Survival curves for the patients were calculated using the Kaplan-Meier method, and analysis was performed using the log-rank test. Prognostic factors were examined by univariate and multivariate analyses using a Cox proportional hazards model. P<0.05 was considered significant. All statistical analyses were performed with the SPSS software package (version 13.0, SPSS, Inc., Chicago, IL).

Results

EpCAM mRNA expression observed by real-time RT-PCR. Analysis of EpCAM mRNA expression in clinical samples showed that 47 of 49 patients (96%) had a higher expression level of EpCAM mRNA in cancerous tissues than in non-cancerous tissues, based on real-time RT-PCR. The mean expression level of EpCAM mRNA expression in tumor tissues was 0.092±0.015 (mean ± SEM), significantly higher than the mean of 0.013±0.001 in the corresponding normal tissues (P<0.0001, Fig. 1).

Table I. The correlation between clinicopathological characteristics and EpCAM expression in immunohistochemistry.

Parameters	No. of patients	EpCAM expression High	Low	P-value[a]
All patients	138	73	65	
Age (years)				0.6498
<62	63	32	31	
≥62	75	41	34	
Gender				0.9970
Male	121	64	57	
Female	17	9	8	
Location				0.4822
Upper	20	13	7	
Midthoracic	84	42	42	
Lower	34	18	16	
Histological grading				0.1600
G1	30	11	19	
G2	69	42	27	
G3	33	16	17	
G4	2	1	1	
Unknown	4			
Histological type				0.1936
Squamous cell carcinoma	129	66	63	
Adenocarcinoma	3	3	0	
Other types	6	4	2	
TNM clinical classification				
T-primary tumor				0.0005
T1	62	45	17	
T2	15	6	9	
T3	53	19	34	
T4	8	3	5	
N-regional lymph node metastasis				0.1199
N0	67	40	27	
N1	71	33	38	
M-distant lymph node metastasis				0.5986
M0	117	63	54	
M1	21	10	11	
Stage grouping				0.0037
I	47	34	13	
II	38	19	19	
III	32	10	22	
IV	21	10	11	
Lymphatic invasion				0.3187
ly (-)	44	26	18	
ly (+)	94	47	47	
Blood vessel invasion				0.0397
v (-)	68	42	26	
v (+)	70	31	39	
Infiltrative growth pattern				0.0015
inf α	36	27	9	
inf ß	89	37	52	
inf γ	13	9	4	

[a]χ^2 test.

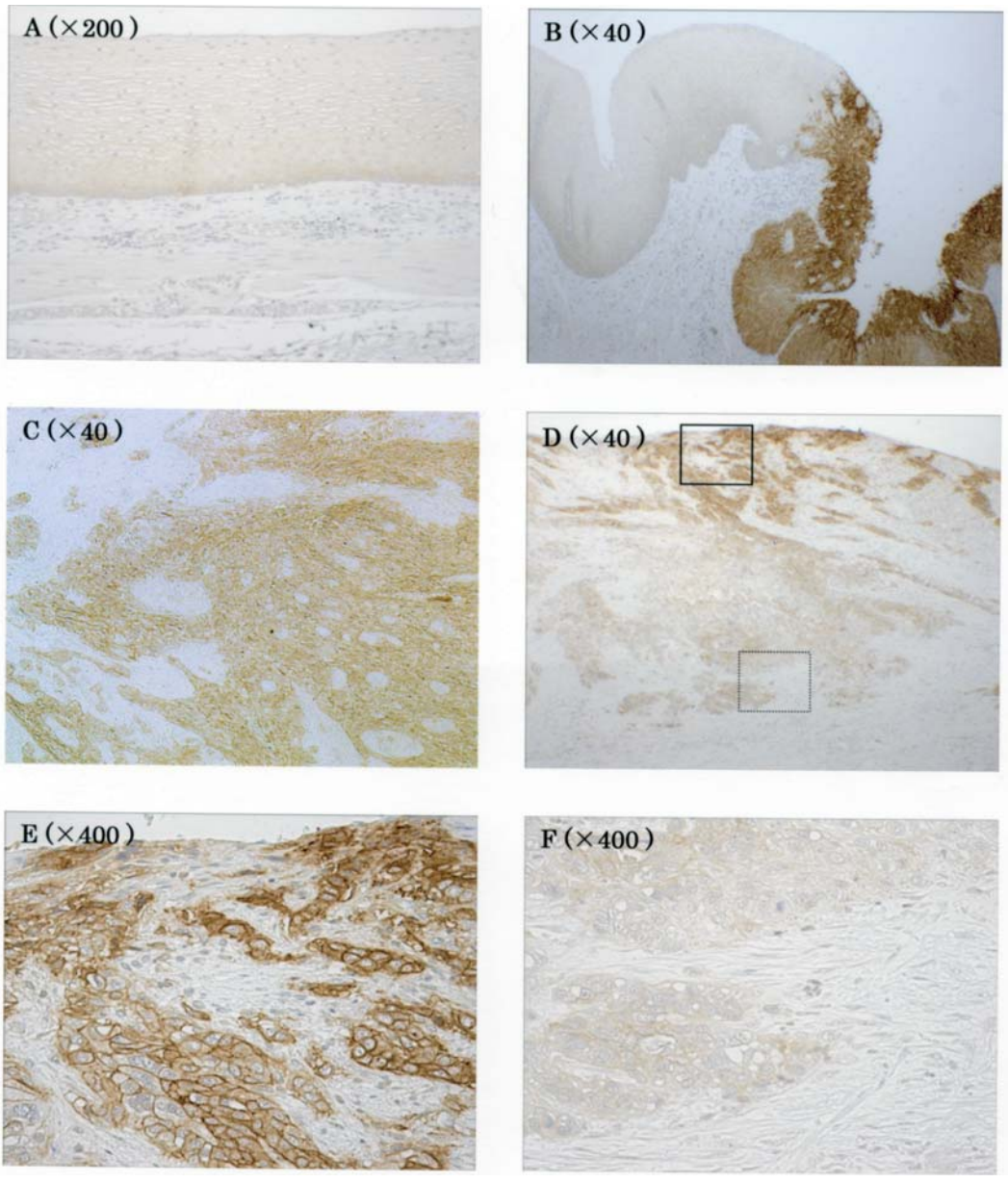

Figure 2. Immunostaining for EpCAM in different tissue sections. (A) Normal esophageal epithelium, in which staining for EpCAM is clearly negative (x200). (B) The border region: EpCAM staining is not detected in the normal esophageal epithelium (left side), whereas strong staining at the plasma membrane is observed in the cancer cells (right side) (x40). (C) Diffuse EpCAM staining in the plasma membrane was identified in advanced cancer tissue (x40). (D) Strong expression of EpCAM protein is apparent in shallow areas of the tumor, but expression is reduced in deeper areas of tumor invasion (x40). (E) High-power view of the shallow area enclosed with a full line in D (x400). (F) High-power view of the invasive front enclosed with a dotted line in D (x400).

Expression of EpCAM protein in esophageal cancer. Staining for EpCAM in the normal esophageal squamous epithelium was clearly negative (Fig. 2A). In cancer cells, EpCAM staining was mainly seen in the plasma membrane, and in border regions only the cancerous lesion stained positive (Fig. 2B). Diffuse staining at the plasma membrane was observed in advanced cancers (Fig. 2C). Some samples showed strong EpCAM expression in shallow areas of the tumor, and reduced expression in deeper areas of the same tumor (Fig. 2D-F). Positive staining for EpCAM was found in 135 (97.8%) of the 138 primary tumor specimens, although heterogeneity was observed in these specimens.

Correlations between EpCAM expression and clinicopathological findings. Patient samples showed a normal distribution of EpCAM expression over different tumor cells. A mean total immunostaining score of >1.5 was considered to indicate high EpCAM expression, and based on this criterion 73 of 138 patients showed high EpCAM expression in the tumor and the other 65 patients showed low expression. The correlation between the clinicopathologic characteristics of the esophageal cancer patients and tumor EpCAM expression is summarized in Table I. EpCAM expression was correlated with tumor depth (P=0.0005), stage (P=0.0037), blood vessel invasion (P=0.0397) and infiltrative growth pattern (P=0.0015). However, there was no significant association between EpCAM expression and other factors, such as age, gender, location, histological grading, histological type, regional lymph node metastasis, distant lymph node metastasis and lymphatic invasion.

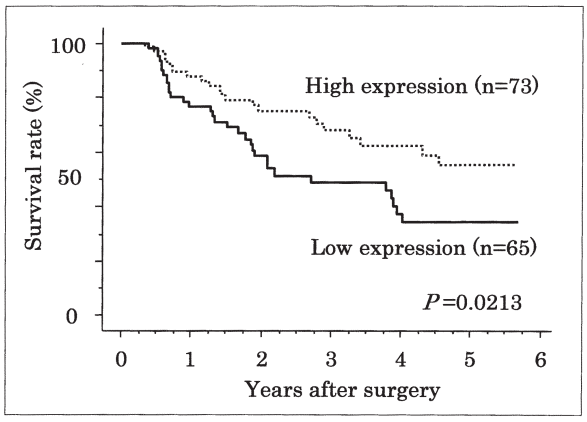

Figure 3. Relationship between overall postoperative survival and EpCAM expression. The survival rates of patients with tumors with high EpCAM expression were significantly higher than those of patients with low EpCAM expression (5-year survival rates: high EpCAM, 55.4%; low EpCAM, 34.4%; P=0.0213).

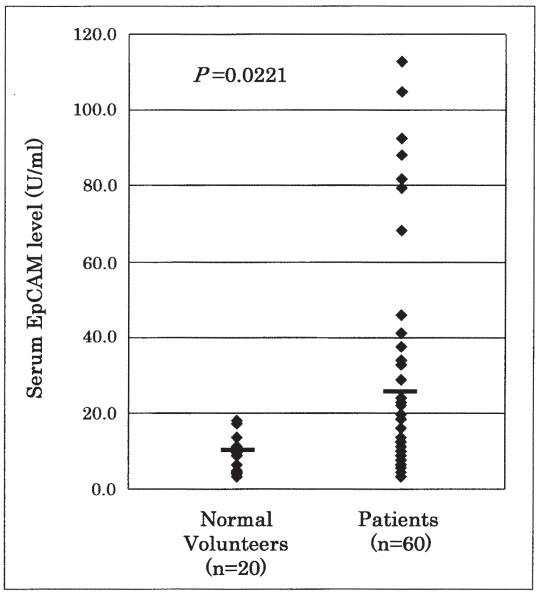

Figure 4. Serum EpCAM levels in normal volunteers and patients with esophageal cancer. Horizontal lines indicate means. The serum EpCAM levels of patients with esophageal cancer were significantly higher than those of normal volunteers (P=0.0221, Mann-Whitney U test).

Prognostic significance of EpCAM expression. The survival rates of patients with tumors showing high EpCAM expression was significantly higher than those of patients with tumors showing low EpCAM expression (P=0.0213; Fig. 3). The 5-year survival rate of patients with high EpCAM expression was significantly higher than that of patients with low EpCAM expression (55.4% vs. 34.4%). However, the tumor EpCAM expression in esophageal cancer patients was not identified as an independent prognostic factor in a multivariate survival analysis using a Cox proportional hazards model (data not shown).

Serum EpCAM levels in patients with esophageal cancer. The preoperative serum EpCAM levels were 23.7±3.5 U/ml (mean ± SEM) in patients with esophageal cancer and

Table II. The correlation between clinicopathological characteristics and serum EpCAM level.

Parameters	No. of patients	S-EpCAM (mean ± SEM[a]) U/ml	P-value
Age (years)			0.2588[b]
<62	27	26.8±5.65	
≥62	33	21.2±4.53	
Gender			0.9411[b]
Male	54	24.4±3.89	
Female	6	1.80±5.73	
Location			0.0803[c]
Upper	8	17.2±5.75	
Midthoracic	35	29.0±5.18	
Lower	17	14.9±4.79	
Histological grading			0.0931[c]
G1	15	10.4±2.10	
G2	26	31.8±6.95	
G3	15	22.6±6.10	
G4	2	27.1±5.39	
Unknown	2		
Histological type			0.2757[c]
Squamous cell carcinoma	52	24.8±4.04	
Adenocarcinoma	5	11.2±3.64	
Other types	3	26.1±3.28	
TNM clinical classification			
T-primary tumor			0.0614[c]
T1	21	23.3±5.76	
T2	5	5.67±1.21	
T3	30	24.5±4.77	
T4	4	42.5±24.0	
N-regional lymph node metastasis			0.4379[b]
N0	26	25.9±5.61	
N1	34	22.1±4.61	
M-distant lymph node metastasis			0.8360[b]
M0	51	24.0±3.94	
M1	9	22.3±8.34	
Stage grouping			0.9925[c]
I	17	20.8±4.95	
II	15	24.0±8.16	
III	19	26.7±7.37	
IV	9	22.3±8.34	
Lymphatic invasion			0.6243[b]
ly (-)	12	21.5±5.30	
ly (+)	48	24.3±4.25	
Blood vessel invasion			0.9477[b]
v (-)	17	22.6±6.50	
v (+)	43	24.1±4.28	
Infiltrative growth pattern			0.4358[c]
inf α	8	27.1±13.5	
inf ß	43	24.4±4.04	
inf γ	9	17.6±7.92	

[a]SEM, standard error of mean; [b]Mann-Whitney's U test; [c]Kruskal-Wallis test.

Table III. Cox proportional hazards analysis.

Risk factor	Reference factor	P-value	Hazard ratio	95% CI
Univariate				
Histological grading	G1, G2, vs. G3, G4	0.0229	2.61	1.14-5.97
Primary tumor (T)	T1 vs. T2, T3, T4	0.0078	5.26	1.55-17-9
Regional lymph node metastasis (N)	Negative vs. positive	0.0006	8.97	2.57-31.3
Distant lymph node metastasis (M)	Negative vs. positive	0.0345	2.78	1.08-7.17
Serum EpCAM level	High vs. low	0.0370	2.88	1.07-7.79
Multivariate				
Histological grading	G1, G2, vs. G3, G4	0.0231	3.03	1.17-7.89
Primary tumor (T)	T1 vs. T2, T3, T4	0.2051	2.35	0.63-8.85
Regional lymph node metastasis (N)	Negative vs. positive	0.0069	6.39	1.66-24.6
Distant lymph node metastasis (M)	Negative vs. positive	0.3989	1.56	0.56-4.36
Serum EpCAM level	High vs. low	0.0074	4.30	1.48-12.5

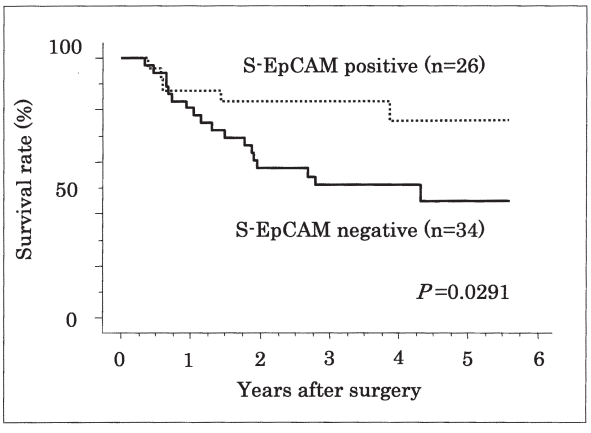

Figure 5. Relationship between overall postoperative survival and serum EpCAM level. The survival rates of patients with high serum EpCAM levels were significantly higher than those of patients with low serum EpCAM levels (5-year survival rates: high EpCAM, 75.8%; low EpCAM, 44.5%; P=0.0291).

9.3±1.1 U/ml in normal volunteers; these levels differed significantly (P=0.0221; Fig. 4). No significant correlation was found between the clinicopathologic characteristics of esophageal cancer patients and serum EpCAM levels in peripheral veins (Table II).

Prognostic significance of serum EpCAM levels. We determined the cutoff value as 13.5 U/ml, which was the median EpCAM level measured from peripheral veins of esophageal cancer patients, to assess the prognostic value of the serum EpCAM level in the peripheral veins. Based on a cutoff value of 13.5 U/ml, patients with esophageal cancer were categorized into two groups: the high serum EpCAM group (n=26) and the low serum EpCAM group (n=34). There were no significant differences in age and sex between these groups. The survival rates of patients with high EpCAM levels in the peripheral vein were significantly higher than those of patients with low serum EpCAM levels (P=0.0291) (Fig. 5). The 5-year survival rate of patients with high EpCAM levels in the peripheral vein was 75.8%, and that of patients with low serum EpCAM levels was 44.5%. Moreover, to clarify whether the serum EpCAM level is a significant prognostic marker, univariate and multivariate survival analyses were performed using a Cox proportional hazards model. These analyses showed that the serum EpCAM level in the peripheral vein is an independent prognostic factor (P=0.0074; hazard ratio 4.30) (Table III).

Discussion

In this study, we investigated the serum EpCAM level and the expression levels of EpCAM mRNA and protein in clinical samples from esophageal cancer patients. EpCAM mRNA expression was significantly increased in tumor tissues compared with normal tissues, EpCAM protein was positively expressed in 97.8% of esophageal cancer patients, and the EpCAM protein level was significantly correlated with tumor invasion, stage grouping, blood vessel invasion and infiltrative growth pattern. Furthermore, the serum EpCAM levels of patients with esophageal cancer were significantly higher than those of normal volunteers, and the serum EpCAM level in the peripheral vein was identified as an independent prognostic factor in esophageal cancer patients.

Expression of EpCAM is found in cancerous lesions in the early phase of carcinogenesis in esophageal cancer patients, and is inversely correlated with tumor progression in these patients. Therefore, it is thought that EpCAM acts as a tumor-specific antigen that stimulates an immunological response, as well as playing a role as an adhesion molecule that suppresses metastasis. These functions may have an effect on prognosis in esophageal cancer patients. Furthermore, a high serum EpCAM level may cause a stronger immunological response against the tumor, and this may account for the identification of the serum EpCAM level as an independent prognostic factor.

EpCAM has been found to be strongly expressed in cancers of various origins (22,37). The expression of EpCAM

in cancerous tissues was anticipated based on pathological studies, and this has recently been confirmed at the mRNA level. Hence, Osta *et al* showed that EpCAM mRNA is significantly overexpressed in primary and metastatic breast cancer tissues (33), and Mitas *et al* reported that EpCAM is useful for discriminating normal esophageal tissue from esophageal adenocarcinoma tissue using real-time RT-PCR (38).

The normal squamous epithelium of the esophagus is clearly negative for EpCAM, while the columnar epithelium in Barrett's esophagus displays a diffuse and low EpCAM expression pattern (39). The expression of EpCAM in esophageal cancer has been reported in preliminary data from Kumble *et al* with high EpCAM expression levels found in four tested adenocarcinomas of the esophagus (16), and Went *et al* reported that 38 of 43 patients (88.4%) with esophageal cancer were EpCAM-positive (22). Our study is the first to suggest a correlation between EpCAM expression and clinicopathological features in esophageal cancer based on a large number of surgical specimens. Correlations between EpCAM expression and clinicopathological features have been reported for cancers of other origins; the expression of EpCAM is correlated with malignant progression of lung cancer (19), breast cancer (23), and gallbladder cancer (25), whereas the EpCAM level has been correlated with suppression of malignancy in ovarian cancer (24), renal cell carcinoma (26) and gastric cancer (27). Hence, the correlation between EpCAM expression and tumor prognosis appears to be bilateral; our data are consistent with the latter investigations showing a correlation with suppression of malignancy.

Our immunohistochemical investigation showed that several patients lost expression of EpCAM at the invasive front. Similar phenomena associated with adhesion molecules have also been observed in previous studies. For example, metastasis of oral squamous cell carcinoma was shown to correlate with a reduction in immunohistochemical staining for desmoplakin and desmoglein at the invasive front (40,41). Furthermore, in oral squamous cell carcinoma, a tendency for reduction of E-cadherin staining at the invasive front has been found, and this reduction was correlated with the mode of invasion (42). In a model system, Basak *et al* showed that EpCAM-mediated adhesion can suppress invasion of tumor-cell grafts in mice (9), and therefore it is plausible that EpCAM-negative cells reduce cell-cell adhesion, thereby promoting invasion and metastasis.

Abe *et al* reported increased EpCAM levels in the serum of approximately 10% of patients with malignant tumors of various tissue origins, suggesting that EpCAM is released from tumor cells into the circulation under certain conditions (43). In our study, the survival rates of patients with high serum EpCAM levels were significantly higher than those of patients with low serum EpCAM levels, and the serum EpCAM level in the peripheral vein was an independent prognostic factor. A high EpCAM level may contribute to a good prognosis in esophageal cancer patients through suppression of malignancy. The levels of other adhesion molecules, such as E-cadherin (44,45) and ICAM-1 (46) have been investigated in several cancers: among patients with advanced ovarian carcinoma, preoperative serum E-cadherin was not correlated with common clinicopathological prognostic variables or with response to chemotherapy and survival (45), and serum ICAM-1 levels did not correlate with malignancy in patients with non-small cell lung carcinoma (46). Furthermore, although a sequential analysis showed that rising serum ICAM-1 levels predicted a short-term fatal outcome, overall serum ICAM-1 levels did not correlate with prognosis (46). Hence, it seems that the level of adhesion molecules in serum does not always reflect the extent of malignant progression.

In contrast to the lack of correlation of the levels of certain adhesion molecules with malignancy, we propose the hypothesis that EpCAM released into the circulation functions as an immunogenic agent and activates an immunologic response against EpCAM-positive tumors. This hypothesis is supported by the recent identification of EpCAM-derived peptides that elicit cellular immune responses restricted by HLA*0201 (47,48), and it is of note that a natural T-cell response against EpCAM has been observed in colorectal cancer patients (49,50). Tajima *et al* reported an epitope from EpCAM that elicited HLA-A*2402-restricted cytotoxic T-lymphocyte responses and investigated the possibility of immunotherapy using the corresponding T-lymphocytes (51). Hence, administration of recombinant EpCAM and induction of cytotoxic T-lymphocyte responses against EpCAM-positive tumors may be useful as a therapy for esophageal cancer. In addition to the potential of EpCAM in targeted immunotherapy, our data show that the serum EpCAM level in the peripheral vein is an independent prognostic factor for survival of patients with esophageal cancer.

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