The overexpression of caveolin-1 and caveolin-2 correlates with a poor prognosis and tumor progression in esophageal squamous cell carcinoma

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Abstract. Caveolin-1 (CAV1) and caveolin-2 (CAV2) are the major structural proteins of caveolae. We investigated the relationship between the clinicopathological factors of esophageal squamous cell carcinoma (ESCC) and the expression of CAV1 and CAV2. CAV1 and CAV2 expression were analyzed by quantitative reverse transcription-polymerase chain reaction (RT-PCR) in 15 esophageal cancer cell lines (TE1-15) and a normal esophageal epithelium cell line (Het-1A). CAV1 and CAV2 expression was examined by RT-PCR and immunohistochemical analysis in 47 ESCC specimens. High levels of *CAV1* and *CAV2* mRNA were detected in TE1-15, but neither CAV1 nor CAV2 mRNA were detected in Het-1A. In the ESCC samples *CAV1* and *CAV2* mRNA expression in the ESCC samples were significantly higher than in the corresponding normal esophageal mucosa (CAV1, P=0.0024; CAV2, P=0.0136). However, we could not find any significant relationship between *CAV1* or *CAV2* mRNA expression and clinicopathological factors. Immunostaining for CAV1 was positive in 13 of 47 patients (27.7%), whereas CAV2 was positive in 22 of 47 patients (46.8%). A significant correlation was observed between CAV1 and CAV2 immunostaining and T factor, lymphatic invasion, vein invasion and differentiation. The patients with positive staining for CAV1 or CAV2 had a significantly shorter survival than those with negative staining (P=0.0105 and 0.0424 for CAV1 and CAV2, respectively). These results suggest that positive staining for CAV1 and CAV2 could be a potentially useful prognostic marker of ESCC.

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Introduction

Esophageal squamous cell carcinoma (ESCC) is one of the most malignant tumors in the gastrointestinal carcinoma family. Patients with ESCC generally have a poor prognosis despite intensive multimodality therapy involving surgery, radiation and chemotherapy. Recent molecular biological studies have revealed that ESCC is caused by the accumulation of multiple genetic defects in dominant oncogenes and tumor suppressors. We have also reported the expression of *surviving* (1), *DFF45/ICAD* (2), *PTTG* (3), *Chfr* (4), *PPARγ* (5), *ERCC3* (6), *PABPC1* (7) and *ACP6* (8) to significantly correlate with the tumor progression and prognosis in patients with ESCC.

Caveolae, which are 50-100 nm protein-coated invaginations of the plasma membrane, play an important role in endocytosis and signal transduction (9,10). Caveolins, the major structural proteins in caveolae, include caveolin-1, -2 and -3. Caveolin-1 (CAV1) and caveolin-2 (CAV2) are coexpressed and form a heterooligomeric complex in many cell types, with particularly high levels in adipocytes (11). CAV1 expression has been reported to increase in cancer of the prostate (12,13), pancreas (14), colon (15), breast (16) and esophagus (17), thus suggesting it to play a positive role in tumor progression. Paradoxically, the CAV1 expression has been observed to decrease in lung (18), colon (19), ovary (20), breast (21,22) and thyroid cancer (23). These data may imply that CAV1 has multiple activities in cancer depending on its interaction with other signaling molecules and the specific cell type or tissue in which it is expressed. Therefore, whether caveolins promote or suppress tumor progression remains controversial.

On the other hand, CAV2 has been reported to have a similar distribution and tissue expression as CAV1, and CAV2 is also an accessory protein that functions in conjunction with CAV1. CAV2 expression has been reported to increase in breast cancer (16), whereas it has been reported to decrease in cancer of lung (18), breast (16,21) and thyroid (23). However, the clinical significance of CAV2 has been less extensively studied than that of CAV1.

A microarray analysis has shown CAV1 and CAV2 to be up-regulated in ESCC cell lines (24). In this study, we

examined CAV1 and CAV2 expression of ESCC by quantitative reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemical analysis in order to clarify the significance of CAV1 and CAV2.

Materials and methods

Cell lines and tissue samples. The esophageal cancer cell lines (TE series) were purchased from the Japanese Cancer Research Resources Bank. The SV40-immortalized esophageal cell line Het-1A was purchased from the American Type Culture Collection. TE esophageal cancer cells were plated in tissue culture dishes and then were grown in RPMI-1640 medium (Sigma) containing 10% fetal bovine serum (JRH Bioscience) at 37˚C in a humidified atmosphere of 95% air and 5% CO_2. Het-1A cells were grown in an LHC-9 serum-free medium (Biofluids, Rockville, MD) in tissue culture dishes at 37˚C in a humidified atmosphere of 95% air and 5% CO_2.

The samples were obtained from 47 patients with primary ESCC who had undergone a radical esophagectomy between 1997 and 2002 at the Department of Surgery II, Nagoya City University Medical School. The study design was approved by the institutional review board of Nagoya City University Medical School, and written consent was obtained from all patients. The tumors were classified according to the Japanese guidelines for clinical and pathological studies on carcinoma of the esophagus. Tissue specimens were collected from 40 males and 7 females, with a mean age of 63.5±7.5 years (range, 45-76 years). All samples were frozen immediately in liquid nitrogen and then were stored at -80˚C until use. All tissue specimens for immunohistochemistry were fixed in formalin and embedded in paraffin. The characteristics of the 47 patients with ESCC are shown in Table I.

RNA extraction and RT-PCR analysis. Total-RNA was extracted from the esophageal cancer tissue and from the corresponding normal esophageal mucosa (taken from the apparently non-cancerous mucosa as far away from the tumor as possible) using the Isogen kit (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. Total-RNA from the cell lines was similarly extracted. The concentration of the total-RNA was adjusted to 200 ng/ml using a spectrophotometer. The reverse transcription reaction was carried out using 1 μg of total-RNA, 0.5 μg of oligo(dT) primer, and Superscript II enzyme (Gibco BRL, Gaithersburg, MD) for 90 min at 42˚C followed by 5 min at 95˚C and 15 min at 72˚C.

TaqMan gene expression assay. Gene expression in all samples was measured by quantitative RT-PCR using an 7700 ABI PRISM Sequence Detector System (Applied Biosystems, Foster City, CA). PCR was performed in a 20-μl reaction mixture containing 10 μl of TaqMan Universal PCR Master Mix (Applied Biosystems), 80 nM of each primer, 2 nM of probe, and 2 μl of cDNA sample. The thermal cycling conditions included an initial denaturation step of 95˚C for 15 sec and 60˚C for 1 min. The relative levels of mRNA expression were calculated from the relevant signals by normalization with the signal for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) expression. PCR primers and fluorogenic probes for all of the target genes and endogenous controls were purchased as Assays-On-Demand (Applied Biosystems). The assays are supplied as a 20x mix of PCR primers and TaqMan minor groove binder 6-FAM dye labeled probes with a non-fluorescent quencher at the 3'-end of the probe. The assays are optimized for use on any ABI PRISM Sequence Detection System using the default machine settings. The assay numbers for *GAPDH*, CAV1 and CAV2 were as follows: Hs99999905_m1 (*GAPDH*); Hs00184697_m1 (*CAV1*); and Hs00184597_m1 (*CAV2*).

Immunohistochemistry. Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded primary human ESCC tissues using 1:150 monoclonal anti-CAV-1 (clone 2297) or 1:50 anti-CAV-2 (clone 65) antibodies (BD Transduction Laboratories, San Jose, CA, USA). Paraffin-embedded sections of tumor were deparaffinized, rehydrated, heat-treated by microwaving in 10 mM citrate buffer for 15 min for antigen retrieval, and cooled to room temperature. Sections were then treated with 0.3% H_2O_2 in methanol for 30 min to neutralize endogenous peroxidases, blocked with non-specific goat serum for 10 min, and incubated with antibody H-100 overnight at room temperature in a humid chamber. Immunoreactive protein was detected with a Dako Envision™ + System, HRP (DAB), and then the sections were counterstained with hematoxylin. The immunostaining of CAV1 and CAV2 was subjectively assessed by two independent investigators (T.A. and H.I.), and discordant results were resolved by consultation with a third investigator (Y.K.). Using light microscopy, the expression of CAV-1 and CAV-2 was scored as follows according to the proportion of positive staining throughout the entire slide: 0, negative or <10%; 1, <33%; 2, 33-67%; and 3, >67%. The status of CAV-1 and CAV-2 immunohistochemical staining was classified as negative for scores of 0 or 1 and positive for scores of 2 or 3.

Statitical analysis. The relative mRNA expression levels (*CAV1*/*GAPDH* and *CAV2*/*GAPDH*) were calculated from the quantified data. The data are expressed as the means ± SD. A statistical analysis was performed using the Stat-View software package (Abacus Concepts, Berkeley, CA). Wilcoxon signed-ranks test was used to evaluate the significance of the difference in the expression of *CAV1*/*GAPDH* and *CAV2*/*GAPDH* mRNA. A correlation test was performed using Pearson's correlation coefficient. Mann-Whitney's U test, Kruskal-Wallis test and Fisher's exact test were used to analyze the association between the mRNA expression or an immunohistochemical analysis and the clinical histopathological parameters of the patients. The survival of ESCC patients after surgery was examined by the Kaplan-Meier method, and the survival time was compared using the log-rank test. A multivariate analysis was performed using Cox's regression model. The P-values were considered significant at the $P<0.05$ level.

Results

Quantitative RT-PCR of CAV-1 and CAV-2. We first investigated the expression of *CAV1* and *CAV2* mRNA in 15 eso-

Table I. Relationship between clinicopathological factors and mRNA expressions of caveolin-1 and caveolin-2.

		Caveolin-1 mRNA (T)		Caveolin-2 mRNA (T)	
	Total		P-value		P-value
Gender					
Male	40	1.966±1.645		3.006±2.641	
Female	7	2.702±2.505	0.4733	3.810±2.794	0.5303
Age					
<65	26	2.459±2.080		3.688±3.113	
≥65	21	1.599±1.218	0.1398	2.429±1.760	0.0951
T factor					
T1	9	0.969±0.433		2.559±1.090	
T2	5	2.787±2.655		4.716±4.851	
T3	21	2.436±1.999		3.046±2.510	
T4	12	1.976±1.363	0.2965	3.027±2.651	0.7511
T1	9	0.969±0.433		2.559±1.090	
T2-4	38	2.337±1.884	0.0549	3.260±2.893	0.9784
N factor					
Negative	13	1.336±0.939		2.721±1.130	
Positive	34	2.358±1.954	0.1536	3.280±3.037	0.5363
Stage					
0	4	0.998±0.426		2.189±0.657	
I	4	0.991±0.550		3.028±1.485	
II	7	2.389±2.154		2.832±1.171	
III	14	2.671±2.206		3.144±2.389	
IV	18	1.970±1.508	0.5054	3.455±3.648	0.8557
0-I	8	0.994±0.455		2.608±1.154	
II-IV	39	2.297±1.876	0.0894	3.231±2.860	0.9099
Lymphatic invasion					
Negative	11	1.743±1.950		3.449±3.460	
Positive	27	2.280±1.894	0.3106	3.133±2.432	0.8595
Unknown	9				
Vein invasion					
Negative	16	1.798±1.415		3.020±2.610	
Positive	22	2.574±2.394	0.5347	3.504±2.933	0.2872
Unknown	9				
Differentiation					
Well	16	2.353±1.971		3.162±2.562	
Moderately	24	2.093±1.812		3.406±2.933	
Poorly	4	0.583±0.362	0.0924	1.372±1.000	0.1419
Unknown	3				

phageal cancer cell lines (TE1-15) and a normal esophageal epithelium cell line (Het-1A) by quantitative RT-RCR. High levels of *CAV1* and *CAV2* mRNA were detected in most of the esophageal cancer cell lines, but neither *CAV1* nor *CAV2* mRNA were detected in Het-1A (Fig. 1a). There was a statistically significant correlation between *CAV1* and *CAV2* mRNA in the cell lines ($\gamma 2=0.848$; $P<0.0001$; Fig. 1b). We next examined the *CAV1* and *CAV2* mRNA expression in 47 pairs of resected ESCC tumors and their corresponding normal esophageal mucosal tissue specimens. Both *CAV1*

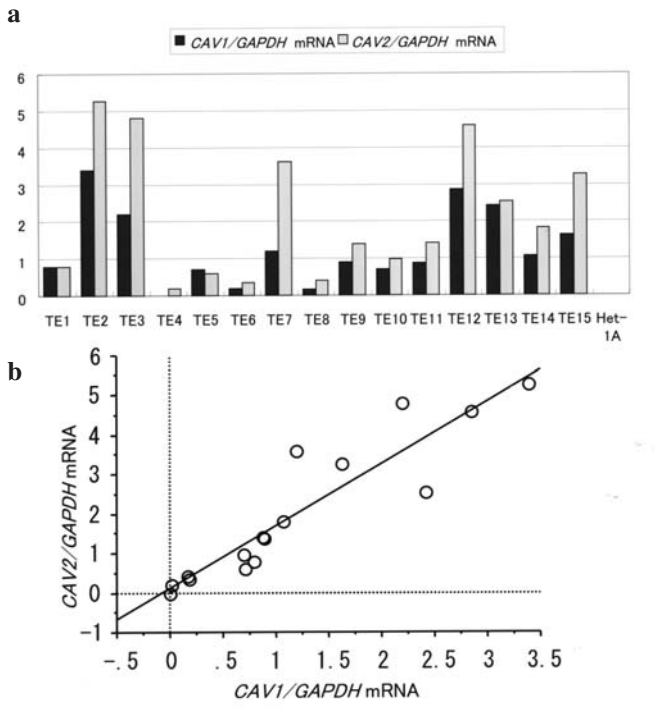

Figure 1. (a) Expression of *CAV1* and *CAV2* mRNA in cell lines. High levels of *CAV1* and *CAV2* mRNA expressions were detected in most of the ESCC cell lines, but neither *CAV1* nor *CAV2* mRNA were detected in the normal esophageal epithelium cell line, Het-1A. (b) The correlation between the *CAV1* and *CAV2* mRNA expression levels in the cell lines. There were significant correlations between *CAV1* and *CAV2* mRNA expression in ESCC cell lines ($\gamma 2$=0.848; P<0.0001).

and *CAV2* mRNA were expressed in the ESCC samples at higher levels than in the corresponding normal esophageal mucosa (CAV1, P=0.0053; CAV2, P=0.0401; Fig. 2). In addition, statistically significant correlations were also observed between *CAV1* and *CAV2* mRNA in normal esophageal mucosal tissue specimens ($\gamma 2$=0.629; P<0.0001; Fig. 3a) and cancerous tissue specimens ($\gamma 2$=0.418; P<0.0001; Fig. 3b).

We examined the relationships between the clinicopathological factors and the expression of *CAV1* or *CAV2* mRNA in ESCC tumors. As a result, *CAV1* and *CAV2* mRNA tended to be found in more aggressive or more advanced tumors, but the difference was not significant (Table I). We also examined the relationship between the expression of *CAV1* or *CAV2* mRNA in ESCC tumors and the survival data. The *CAV1* and *CAV2* mRNA expressions were divided into two groups (a high expression and low expression). However, we could not find any significant relationships between *CAV1* or *CAV2* mRNA expression and survival data (data not shown).

Immunostaining for CAV-1 and CAV-2. We further studied the expression of CAV1 and CAV2 protein in the ESCC tissue specimens by immunohistochemistry. Typical ESCC cells showed diffuse cytoplasmic staining, and in cases with intense staining, both the cell membrane and the cytoplasm were stained for CAV1 and CAV2 (Fig. 4b and e). Positive staining for CAV1 and CAV2 was also observed adipocytes, stromal cells in smooth muscle cells (Fig. 4e and f). In contrast, normal esophageal epithelium showed negative staining for CAV1 and CAV2 (Fig. 4a and d). Immunostaining for CAV1 and CAV2 was positive in 13 (27.7%) and 22 (46.8%) of 47 patients, respectively. Table II shows the relationship between the immunostaining findings for CAV1 or CAV2 and the clinicopathological factors. Immunostaining for CAV1 and CAV2 did not differ according to age, lymph node metastasis or stage. Both CAV1 and CAV2 immunostaining showed a significantly positive correlation with T factor, lymphatic invasion and differentiation. Both CAV1 and CAV2 proteins were overexpressed in the more aggressive ESCC tumors with local invasiveness and lymphatic invasion. Furthermore, these two proteins were more highly expressed in well-differentiated than in poorly differentiated ESCC tumors. In addition, CAV2 immunostaining was also significantly related with vein invasion and it was significantly more frequent in females than in males.

We investigated the correlation between immunostaining for CAV1 and CAV2 and survival in ESCC patients after surgery (median follow-up, 26.2 months). The patients with positive staining for CAV1 had a significantly shorter survival after surgery than the patients with negative staining [15.7±2.5 (n=13) vs. 21.1±1.5 (n=34) months, respectively; P=0.0105 by

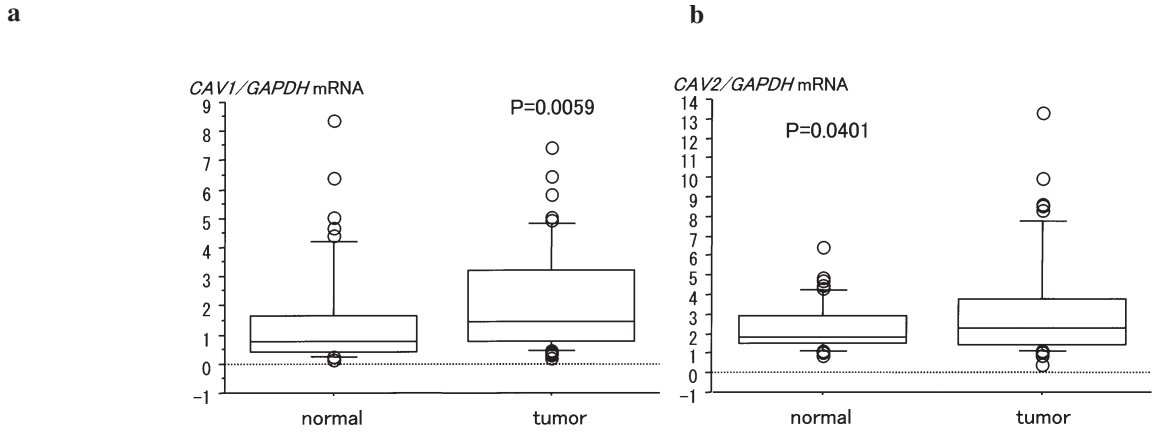

Figure 2. Comparison of the expression of *CAV1* (a) and *CAV2* (b) mRNAs in ESCC tissues and their corresponding normal esophageal mucosal tissues. *CAV1* and *CAV2* mRNA expression in ESCC were significantly higher than those in the corresponding normal esophageal mucosa (P=0.0059 and 0.0401 for CAV1 and CAV2, respectively, according to Wilcoxon signed-ranks test).

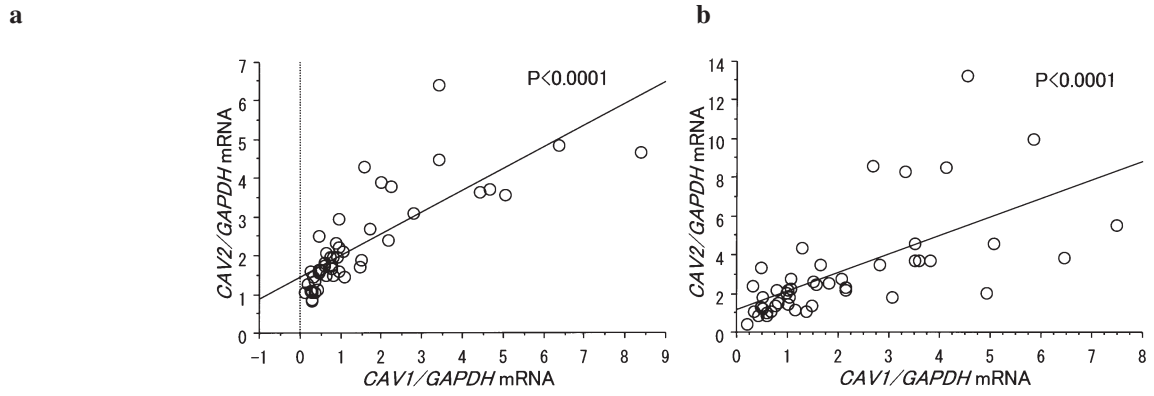

Figure 3. Correlation between the *CAV1* and *CAV2* mRNA expression levels in normal esophageal mucosal tissues (a) and ESCC tissues (b). Statistically significant correlations were observed between *CAV1* and *CAV2* mRNA expression in normal esophageal mucosal tissues ($\gamma 2$=0.629; P<0.0001) and cancerous tissues ($\gamma 2$=0.418; P<0.0001).

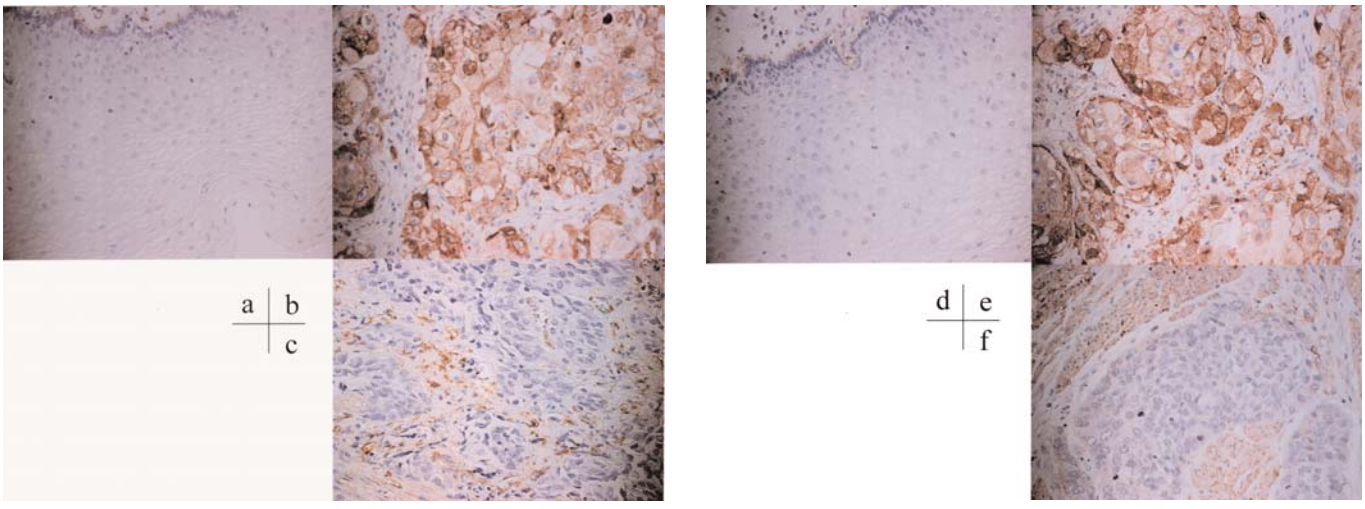

Figure 4. Representative immunostaining for CAV1 and CAV2 (x400). (a) Negative staining of CAV1 in normal esophageal mucosa. (b) Positive staining in CAV1 in tumor cells. (c) Negative staining for CAV1 in tumor cells but strong staining of the stroma. (d) Negative staining for CAV2 in normal esophageal mucosa. (e) Positive staining for CAV2 in tumor cells. (f) Negative staining for CAV2 in tumor cells but strong staining for smooth muscle cells.

log-rank test; Fig. 5a]. In addition, the patients with positive staining for CAV2 had a significantly shorter survival after surgery than the patients with negative staining [17.3±2.1 months (n=22) vs. 31.3±3.3 months (n=25); P=0.0424 by log-rank test; Fig. 5b].

A univariate analysis showed that, among the clinicopathological factors, the extent of primary tumor (risk ratio, 6.463; P=0.0123), lymph node metastasis (risk ratio, 5.532; P=0.0214), vein invasion (risk ratio, 4.323; P=0.0241), immunostaining for CAV1 (risk ratio, 2.842; P=0.0155) were all statistically significant prognostic factors, whereas immunostaining for CAV2 was not found to be a prognostic factor in univariate analysis (Table III). The multivariate analysis revealed that positive staining for CAV1 was an independent prognostic factor (risk ratio, 3.858; P=0.0164), as well as lymph node metastasis (risk ratio, 10.238; P=0.0303) (Table IV).

Discussion

Caveolins are the major structural proteins of caveolae and have been reported to interact with various intracellular signaling molecules including epidermal growth factor receptor (25), HER2 (21), c-myc (26), transforming growth factor-ß/SMAD (27) and the Wnt pathway (28). The expression and function of CAV1 has been examined in several cancers such as prostate (12,13), pancreas (14), colon (15,19), lung (18), ovary (20), breast (16,21,22) and thyroid cancer (23), whereas just a few investigations on CAV2 have so far been reported in breast (16,21), lung (18) and thyroid cancer (23). To the best of our knowledge, only one report has been released regarding the relationship between the immunostaining for CAV1 and the prognosis of ESCC (17). However, CAV2 expression has not yet been examined in ESCC. In this study we evaluated the expression of both CAV1 and CAV2 in ESCC, not only by quantitative RT-PCR but also by immunostaining.

We thus demonstrated that the expressions of both *CAV1* and *CAV2* mRNA were significantly higher in the tumor tissues than in the corresponding normal tissues, in addition to the ESCC cell lines. However, they did not correlate with the clinicopathological factors and thus could not be prognosis factors. The expression of both CAV1 and CAV2 protein significantly correlated with several clinicopatho-

Table II. Relationship between clinicopathological factors and immunostaining for CAV1and CAV2.

		CAV1			CAV2		
	Total	Negative 34	Positive 13	P-value	Negative 25	Positive 22	P-value
Gender							
Male	40	31	9		24	16	
Female	7	3	4	0.0805	1	6	0.0400
Age							
<65	26	17	9		14	12	
≥65	21	17	4	0.3300	11	10	>0.9999
T factor							
T1	9	9	0		8	1	
T2	5	4	1		3	2	
T3	21	13	8		7	14	
T4	12	8	4	0.1775	7	5	0.0430
T1	9	9	0		8	1	
T2-4	38	25	13	0.0468	17	21	0.0252
N factor							
Negative	13	11	2		9	4	
Positive	34	23	11	0.3007	16	18	0.2071
Stage							
0	4	4	0		4	0	
I	4	4	0		3	1	
II	7	4	3		2	5	
III	14	10	4		7	7	
IV	18	12	6	0.3845	9	9	0.1904
0-I	8	8	0		7	1	
II-IV	42	26	13	0.0855	18	21	0.0516
Lymphatic invasion							
Negative	11	11	0		11	0	
Positive	27	18	9	0.0378	10	17	0.003
Unknown	10	-	-		-	-	
Vein invasion							
Negative	16	15	1		14	2	
Positive	22	14	8	0.0525	7	15	0.0009
Unknown	10	-	-		-	-	
Differentiation							
Well	16	8	8		3	13	
Moderately	24	20	4		16	8	
Poorly	4	4	0	0.0298	4	0	0.0016
Unknown	3	-	-		-	-	

logical factors including T factor, lymphatic invasion, and differentiation. Positive staining for CAV1 or CAV2 had a significant correlation with a poor survival after surgery, and both of CAV1 and CAV2 protein expression could thus be prognosis factors according to the log-rank test. These results implied that both CAV1 and CAV2 might therefore play a role in the development and differentiation of ESCC. Our results were similar to those of previous reports which

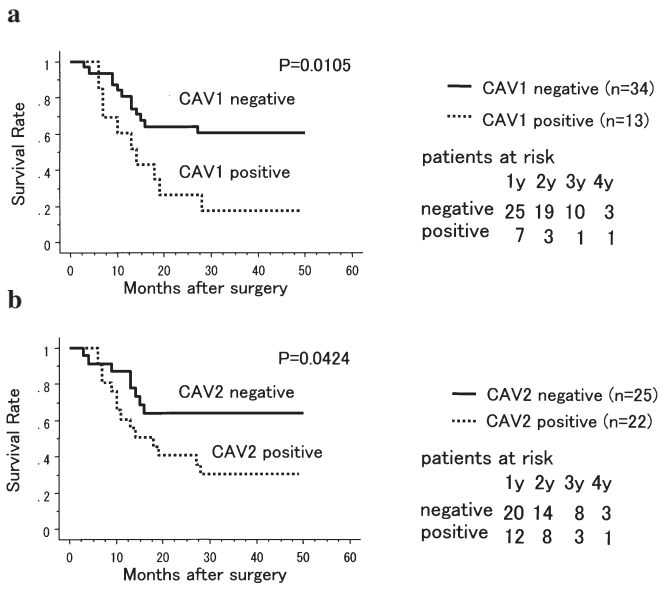

Figure 5. Survival rate of patients with ESCC according to CAV1 and CAV2 immunostaining. (a) Patients with positive staining for CAV1 had a significantly shorter survival after surgery than patients with negative staining for CAV1 (P=0.0105). (b) Patients with positive staining for CAV2 had a significantly shorter survival after surgery than patients with negative staining for CAV2 (P=0.0424).

Table III. Univariate analysis.

Parameter	Risk ratio	95% CI[a]	P-value
Age at surgery			
<65	1		
≥65	1.485	0.614-3.440	0.3557
Gender			
Female	1		
Male	1.224	0.362-4.138	0.7449
Histlogical grade			
Moderately/poorly	1		
Well	1.775	0.720-4.373	0.2124
Primary tumor			
T1-2	1		
T3-4	6.463	1.499-27.873	0.0123
Lymph node metastasis			
Negative	1		
Positive	5.532	1.288-23.766	0.0214
Lymphatic invasion			
Negative	1		
Positive	7.364	0.965-56.220	0.0542
Vein invasion			
Negative	1		
Positive	4.323	1.211-15.436	0.0241
Immunostaining for CAV1			
Negative	1		
Positive	2.842	1.220-6.621	0.0155
Immunostaining for CAV2			
Negative	1		
Positive	2.377	0.994-5.684	0.0515

[a]CI, confidence interval.

described the CAV1 expression to increase in cancer of prostate (12,13), pancreas (14), colon (15), breast cancer (16) and esophagus (17). Overexpression of CAV2 has also been reported in breast cancer (16). Our findings suggest that not only CAV1 but also CAV2 may thus play an important role in tumor progression. However, the precise mechanism of tumor progression mediated by caveolin remains to be eludicated. Caveolae was proposed to regulate the cellular signaling pathways, because many receptors and signal transduction molecules are concentrated within the caveolae. Especially CAV1 is considered to play a role in both cell survival and tumor progression, by activating the PI3-K/Akt pathway (29) and interacting with GTP binding proteins (30) and C-Src (31). The functions of caveolin might thus be regulated by these intracellular signaling pathways.

In our study, positive staining for CAV1 and CAV2 in tumor tissue was higher in females than in males. In particular, positive staining in females was significantly high in CAV2. This suggests that the expression of caveolins, including CAV1 and CAV2, may be related to gender, because E2 (17-ß-estradiol) has been reported to stimulate CAV1 and CAV2 protein expression in smooth muscle cells (32).

In this study, we also demonstrated an increased expression of CAV1 and CAV2 both at mRNA level and at the protein level, and moreover a significant correlation between the CAV1 and CAV2 expression in both the cell lines and resected ESCC samples. Previously, CAV2 was not implicated to play an important role in cancer, while CAV2 was thought to be an accessory protein of CAV1. However, considering that CAV1 and CAV2 are the main structural components of caveolae and they form a heterooligomeric complex (11), our findings suggest that CAV2 may therefore also play an important role in intracellular signaling as well as CAV1. In resected ESCC samples, the expression of CAV1 and CAV2 protein was significantly related with clinicopathological factors. Therefore, the overexpression of both CAV1 and CAV2 may contribute to tumor progression in ESCC.

This is the first study to demonstrate the clinical significance of both CAV1 and CAV2 expression in ESCC. In particular the expression of CAV2 has not been previously examined in ESCC. Our results showed that immunostaining for CAV1 and CAV2 may be more effective than mRNA expression for determining the malignant potential of ESCC. We showed that positive immunostaining for CAV1 and CAV2 could be prognostic factors in ESCC. Furthermore, the multivariate analysis showed positive immunostaining for

Table IV. Multivariate analysis including CAV1.

Parameter	Risk ratio	95% CI[a]	P-value
Primary tumor			
T1-2	1		
T3-4	3.723	0.767-18.071	0.1029
Lymph node metastasis			
Negative	1		
Positive	10.238	1.247-84.033	0.0303
Vein invasion			
Negative	1		
Positive	2.914	0.790-10.752	0.1084
Immunostaning for CAV1			
Negative	1		
Positive	3.858	1.281-11.614	0.0164

[a]CI, confidence interval.

CAV1 to be an independent prognosis factor, although that for CAV2 was not. Since positive staining of CAV1 might be a more significant prognostic marker than CAV2, the assessment of positive staining of CAV1 and CAV2 is a candidate prognostic marker for patients with ESCC. Caveolins including CAV1 and CAV2 might therefore be new therapeutic targets in ESCC.

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