Endoglin (CD105) Is a Useful Marker for Evaluating Microvessel Density and Predicting Prognosis in Esophageal Squamous Cell Carcinoma

TOSHIHIDE SAKURAI, HIROSHI OKUMURA, MASATAKA MATSUMOTO, YASUTO UCHIKADO, TETSUHIRO OWAKI, YOSHIAKI KITA, TETSURO SETOYAMA, ITARU OMOTO, YUKO KIJIMA, SUMIYA ISHIGAMI and SHOJI NATSUGOE

Department of Digestive Surgery, Breast and Thyroid Surgery Graduate School of Medical Sciences, Kagoshima University, Sakuragaoka, Kagoshima, Japan

Abstract. *Background: Angiogenic molecular markers such as vascular endothelial growth factor and tumor microvessel density reflect prognosis of human cancers. The present study clarified the usefulness of endothelial marker endoglin (CD 105) by assessing microvessel density in esophageal squamous cell carcinoma (ESCC). Materials and Methods: We immunohistochemically investigated CD105, CD31, and vascular endothelial growth factor-A VEGF-A expression in primary esophageal squamous cell carcinoma specimens from 142 patients. Results: Microvessel density was 35.9±21.2 for CD105 and 46.3±25.4 for CD31. CD105 microvessel density was significantly associated with tumor length, tumor invasion depth, lymph node metastasis, stage, lymphatic invasion, venous invasion, and VEGF-A expression; its correlation with almost all clinicopathological parameters was stronger than CD31 microvessel density. And significantly better prognosis was achieved in patients with low, compared to high CD105, microvessel density. Conclusion: CD105 microvessel density reflected the degree of angiogenesis and prognosis in patients with esophageal squamous cell carcinoma.*

Esophageal squamous cell carcinoma (ESCC) has greater malignant potential and poorer prognosis than other gastrointestinal cancers. ESCC forms advanced tumors and hematogeneous or lymphatic metastasis can occur even after curative surgery, adversely affecting prognosis. Angiogenesis is one of the most important factors in the formation of these advanced tumors and recurrent disease. Aberrant angiogenesis, the formation of new blood vessels, is vital for solid tumors and contributes to malignant potential by facilitating invasion and metastasis (1). Since these newly-formed blood vessels have good permeability, cancer cells can easily pass though their endothelium and metastasize through the circulation (2).

Because cancers need an increasing supply of nutrients and oxygen to develop, tumors release vascular proliferation factors like vascular endothelial growth factor (VEGF) to promote angiogenesis and to satisfy demand (3, 4). Therefore, assessing the expression of such angiogeneic molecular markers and measuring tumor microvessel density (MVD) induced by these growth factors is thought to reflect prognosis and metastatic potential in human cancers (5-7). Various reports have demonstrated the usefulness of VEGF expression in predicting prognosis (8, 9). However, Gadducci *et al*. reported that VEGF expression alone could not predict prognosis of advanced ovarian cancer (10). Moreover, in gastric cancer, differences in VEGF expression did not influence the post-operative survival rate (11). Among the VEGF family, it has been assumed that VEGF-A and C contribute to the vascular endothelium formation. Recent knowledge however elucidated that VEGF-C was mainly concerned with lymphangiogenesis (12). In addition to VEGF expression, pan-endothelial markers, including von Willebrand factor (factor VIII), CD31, and CD34, have been used for neovascular evaluation to determine MVD. Recently, endoglin (CD105) has also been used to evaluate MVD; this marker is considered an important advance, as it is thought to identify only new blood vessels induced by the tumor and not normal vessels (13, 14).

The aim of this retrospective study was to examine the relationship between CD105 expression, CD31 expression, VEGF-A expression and clinicopathological findings in surgical specimens of ESCC. We compared CD105 with CD31 in terms of application in predicting outcome.

Correspondence to: Hiroshi Okumura, MD, Ph.D., Department of Digestive Surgery, Breast and thyroid surgery Graduate School of Medical Sciences, Kagoshima University, Sakuragaoka 8-35-1, Kagoshima 890-8520, Japan. Tel: +81 992755361, Fax: +81 992657426, e-mail: hokumura@m.kufm.kagoshima-u.ac.jp

Key Words: Endoglin (CD105), CD31, vascular endothelial growth factor, esophageal cancer.

Patients and Methods

Study groups. This study enrolled 142 consecutive patients (130 men and 12 women) with ESCC who underwent curative surgery at the Kagoshima University Hospital between 1996 and 2003. All patients underwent curative esophagectomy consisting of a laparotomy and right thoracotomy with three-field lymphadenectomy. To examine the degree of lymph node metastasis, all patients received ultrasound, endoscopic ultrasonography and computed tomography of the neck, thorax and abdomen before surgery. None of them underwent endoscopic mucosal resection, palliative resection, preoperative chemotherapy or radiotherapy, and none had synchronous or metachronous multiple cancers in other organs. Patient age ranged from 38 to 86 years (mean=64.9). Specimens of the tumor were collected from the patients at operation after informed consent had been obtained and the study had been approved by the institutional review board of our university.

Clinicopathological findings were based on the tumor-node-metastasis classification for esophageal carcinoma from the International Union Against Cancer (15). Histologically, all patients had squamous cell carcinoma; well-differentiated in 41, moderately-differentiated in 76, and poorly-differentiated in 25. Depth of tumor invasion was assessed as pathological (p) T1 in 57 (40.1%), pT2 in 22 (15.5%), pT3 in 54 (38.0%), and pT4 in 9 (6.4%). Lymph node metastasis (pN1) was found in 81 (57.0%) of the 142 patients and distant metastasis (pM1) in 27 (19.0%). Lymphatic and venous invasion were found in 69.7% (99/142) and 57.0% (81/142) of patients, respectively. All of the M1 tumors demonstrated distant lymph node metastases. All patients were followed-up after discharge with a radiographic examination every 1-3 months, computed tomography every 3-6 months, and ultrasonography every 6 months. Follow-up data after treatment were collected from all patients with a median follow-up period of 41 months (range 1-137 months). The clinicopathological features of the study group are summarized in Table I.

Immunohistochemistry. Tumor samples were fixed with 10% formaldehyde in phosphate buffer saline (PBS), embedded in paraffin, and sectioned into 4-μm thick slices. They were de-paraffinized in xylene and dehydrated in graded ethanol. Sections did not need manipulation for antigen retrieval. The endogenous peroxidase activity of specimens was blocked by immersing the slides in a 3% H_2O_2 solution for 30 min. After washing three times with PBS for 5 min each, the sections were treated with 3% bovine serum albumin (BSA) for 30 min at room temperature. The blocked sections were incubated for 2 h at room temperature with the anti-human CD105 mouse monoclonal antibody (Dako Corp., Carpinteria, CA, USA) 1:200, anti-CD31 mouse monoclonal antibody (Dako) 1:50, and purified rabbit polyclonal antihuman VEGF (A-20; Santa Cruz Biotechnology, Inc., CA, USA) diluted 1:200, followed by staining with a streptavidin-biotin peroxidase kit (Nichirei, Tokyo, Japan). The sections were washed three times in PBS for 5 min each time and the immune complex was visualized by incubating the sections with diaminobenzidine tetrahydrochloride (DAB•4HCl). Sections were then counterstained with hematoxylin.

Evaluation of CD105 and CD31. Immunostaining for CD105 and CD31 was used to determine MVD (CD105-MVD and CD31-MVD, expressed in terms of vessel count). Vessel count was assessed by light microscopy in those areas of the tumor containing the highest numbers of capillaries and small venules at the invasive edge. These highly vascular areas were identified by scanning tumor sections at low power (×40 and ×100). After six areas were identified, vessel count was performed in a ×200 field, and the average of six areas was determined as MVD. As described by Weidner *et al*., identification of a vessel lumen was not necessary for a structure to be defined as a vessel (16).

Evaluation of VEGF-A. Five fields were viewed at low power (×40 and ×100) and predominant VEGF intensity was determined based on a tumor scale of 0 to 3+. We defined a score of 3+ as the strongest stain (positive control) and 0 as no detectable stain (negative control) as reported previously (17). We defined tumors with a score of 3 as positive, and those with a score of 0-2 as negative.

Statistical analysis. Data were analyzed using the χ^2 test or Student's *t*-test for statistical significance. The Kaplan-Meier method was used for survival analysis, and differences were estimated with the log rank test. The prognostic factors were examined by univariate and multivariate analyses (Cox proportional hazards regression model). The *p*-values in this study were two-sided and a *p*-value of <0.05 was considered to indicate statistical significance. All statistical analyses were performed using the software package StatView™ version 5.0 (Abacus Concepts, Berkeley, CA, USA). Results are given as mean±SD, other than where indicated.

Results

Expression of CD105 and CD31 in ESCC. CD105 (Figure 1A) and CD31 (Figure 1B) were detected in blood endothelial cells. Comparison of CD105 and CD31 expression revealed fewer vessels with better contrast for CD105 than for CD31. CD31-MVD was significantly greater than CD105-MVD (mean±SD: 46.3±25.4 *vs*. 35.9±21.2; $p<0.0001$).

Relationship between the expression of CD105-MVD, CD31-MVD, and clinicopathological findings. Tumors were divided into low-MVD and high-MVD groups, according to the median MVD values of 34.7 for CD105 and 44.4 for CD31. High CD105-MVD was significantly associated with the following parameters: tumor length ($p=0.001$), tumor invasion depth ($p<0.0001$), lymph node metastasis ($p<0.0001$), distant metastasis ($p=0.005$), stage ($p<0.0001$), lymphatic invasion ($p=0.0001$), and venous invasion ($p<0.0001$). High CD31-MVD was significantly associated with the following clinicopathologic parameters: tumor length ($p=0.01$), tumor invasion depth ($p=0.0002$), lymph node metastasis ($p=0.03$), stage ($p=0.0003$), lymphatic invasion ($p=0.04$), and venous invasion ($p=0.03$). Hence CD105-MVD correlated more with clinicopathological parameters than CD31-MVD (Table I).

Correlation between CD105-MVD, CD31-MVD, and VEGF-A. The expression of VEGF-A was examined as a marker of angiogenesis. VEGF-A was detected in the cytoplasm of ESCC cells (Figure 1C). Seventy-six patients (53.5%) were

Table I. *Correlations of CD105 and CD31 expression with clinicopathologic findings.*

	CD105 expression			CD31 expression		
	Low <34.7 (n=71)	High >34.7 (n=71)	*p*-Value	Low <44.4 (n=71)	High >44.4 (n=71)	*p*-Value
Gender						
Male	61	66	NS	65	65	NS
Female	7	5		6	6	
Age	64±9	65±9	NS	64±8	65±9	NS
Tumor length	4.2±5.3	5.4±3.8	0.001	4.3±5.0	5.2±4.4	0.01
Histology						
Well	17	24	NS	17	24	NS
Mod.	45	31		45	31	
Poor	9	16		9	16	
pT						
T1	44	13	<0.0001	42	15	0.0002
T2	11	11		9	13	
T3	11	43		17	37	
T4	5	4		3	6	
pN						
N0	42	19	<0.0001	37	24	0.03
N1	29	52		34	47	
pM						
M0	64	51	0.005	60	55	NS
M1	7	20		11	16	
pStage						
I	31	6	<0.0001	29	8	0.0003
II		24	19		22	21
III	9	26		9	26	
IV	7	20		11	16	
Lynphatic invasion						
Negative	32	11	0.0001	27	16	0.04
Positive	39	60		44	55	
Venous invasion						
Negative	43	18	<0.0001	37	24	0.03
Positive	28	53		34	47	

NS: Not significant.

Table II. *Correlations of CD105 expression and CD31 expression with VEGF-A.*

	CD105 expression			CD31 expression		
VEGF-A	Low (n=71)	High (n=71)	*p*-Value	Low (n=71)	High (n=71)	*p*-Value
Negative	45	21	<0.0001	41	25	0.007
Positive		26	50		30	46

considered as having a positive result. Both CD105-MVD and CD31-MVD were significantly associated with VEGF-A expression. VEGF-A expression was stronger in the tumors with high MVD than in those with low MVD ($p<0.0001$ *vs.* $p=0.007$). Furthermore, VEGF-A expression correlated more with CD 105-MVD than with CD31-MVD (Table II).

Post-operative recurrence pattern according to CD105 and CD31 expression. Among the study group, 28 patients had hematogenous recurrence and 34 patients had lymph node recurrence in this follow up period. In the high CD105-MVD group, hematogenous or lymph node metastasis occurred in 19 and 20 patients, respectively. In the high CD31-MVD

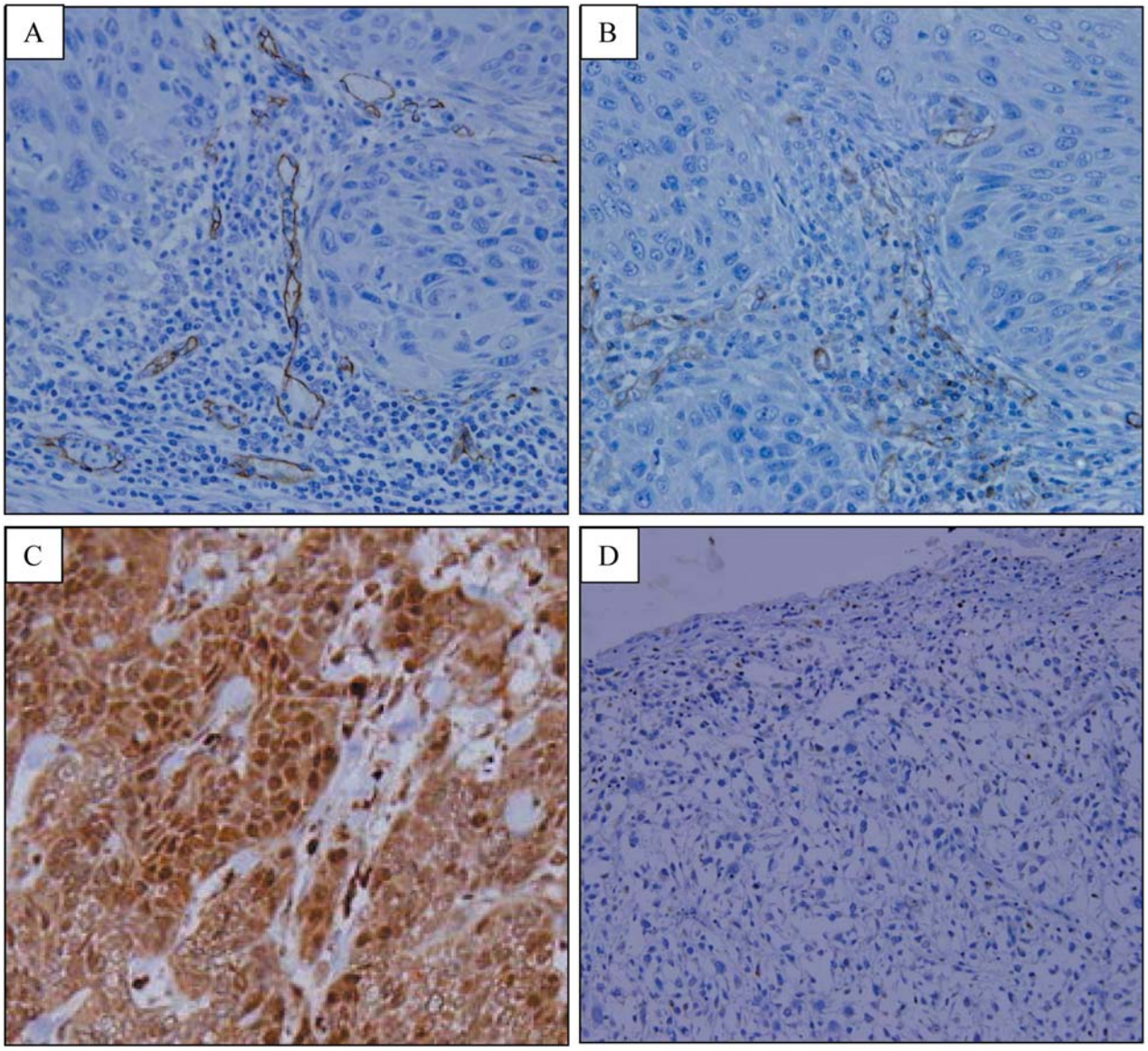

Figure 1. *Expression of microvessels in the tissue of esophageal squamous cell carcinoma. (A), CD105 expression in ESCC (×200). (B): CD31 expression in ESCC (×200). (C): strong expression of VEGF-A detectable in cytoplasmic regions (×200). (D): negative expression of VEGF-A.*

group, hematogenous or lymph node metastasis occurred in 14 and 16 patients, respectively (Table III). Hematogenous metastasis was more frequent in the high CD105-MVD group than in the high CD31-MVD group (p=0.03).

Relationship between expression of CD105, CD31 and prognosis. CD105-MVD was significantly associated with overall survival (p=0.001) and disease-free survival (p=0.0044). On the other hand, CD31-MVD was not associated with overall survival (p=0.35) or disease-free survival (p=0.28). Patients with high CD105-MVD had poor prognosis (Figure 2).

Univariate and multivariate analysis of survival. Univariate analysis showed that the following factors were significantly related to post-operative survival: depth of tumor invasion, lymph node metastasis, lymphatic invasion, venous invasion, and CD105-MVD. Multivariate regression analysis indicated that tumor invasion depth, lymphatic invasion, and venous invasion were independent prognostic factors (Table IV).

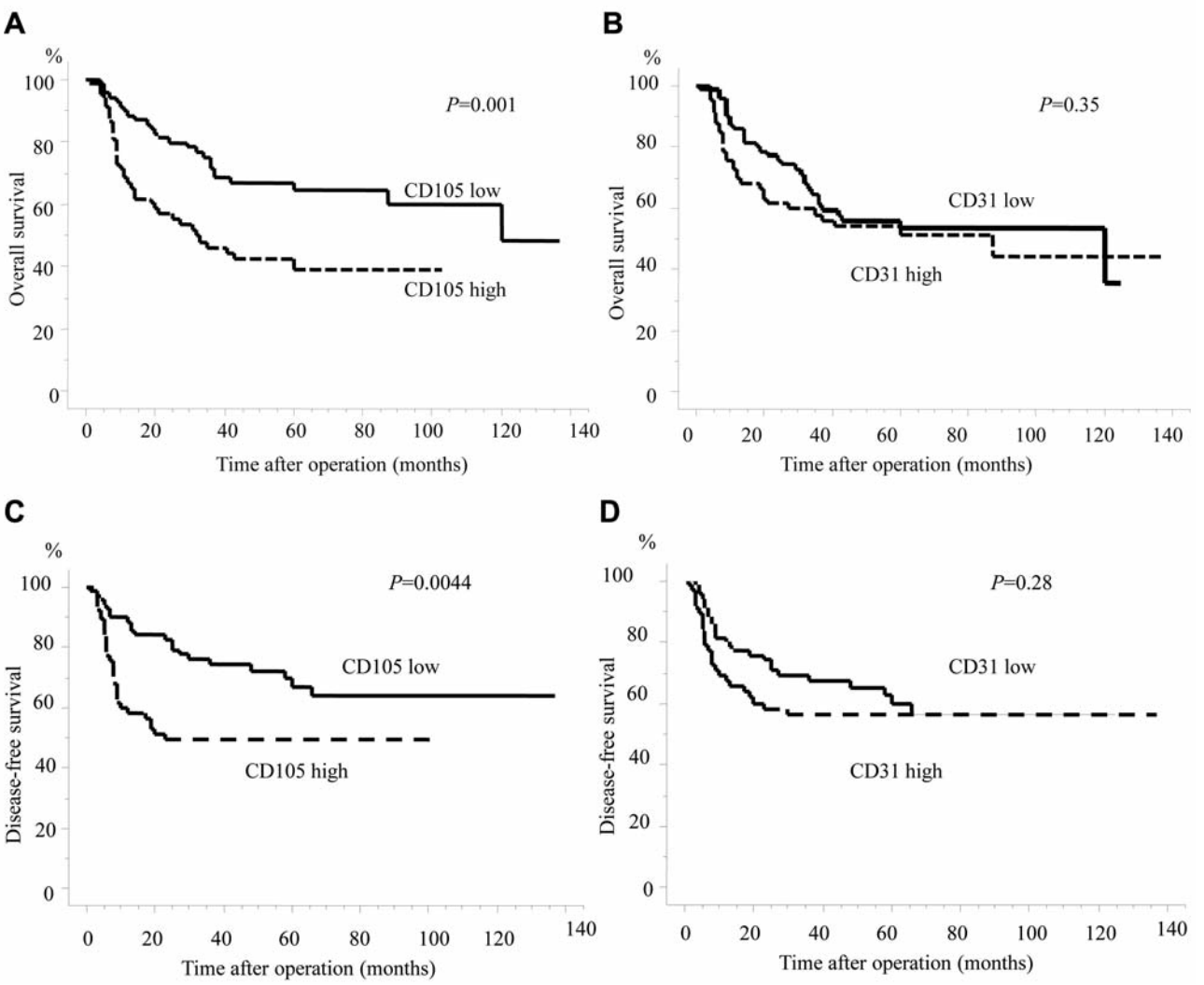

Figure 2. *Overall survival (A, B) and disease-free survival (C, D) curves according to the expression of CD105 and CD31. (A) The patients with low CD105 expression tumors had longer overall survival than those with high CD105 expression tumors (p=0.001). (B)There was no significant difference of overall survival between the patients with high CD31 expression and the patients with low CD31 expression. (C) The patients with low CD105 expression tumors had significantly longer disease-free survival than those with high CD105 expression tumors. (D) There was no significant difference of disease-free survival between the patients with high CD31 expression and the patients with low CD31 expression.*

Discussion

The relationship between tumor properties and MVD has been discussed in reports in which factor VIII, CD31, and CD34 were used to evaluate MVD. Increased MVD assessed using factor VIII was related to tumor differentiation and lymph node metastasis, but not to tumor depth in breast cancer (17). In esophageal cancer, increased factor VIII-MVD was significantly associated with tumor depth, intramural metastasis, and stage. Furthermore, factor VIII-MVD was an independent predictor of poor prognosis (18). The survival rate was significantly poorer in patients with high-factor VIII-MVD than in those with low-factor VIII-MVD (5). MVD assessed with CD31 was correlated significantly with lymph node metastasis, stage, and depth of tumor invasion, as well as with stage and survival rate (19, 20). Regarding CD34, MVD assessed using this factor was an independent prognostic factor for overall survival (21). High CD34-MVD associated with stage, tumor size, and lymph node metastasis was an independent predictor of survival in lymph node-negative cases (22).

Accordingly, the results regarding MVD vary considerably. This might have two reasons: methodological differences in assessing MVD and differences in antibodies used to

Table III. *Comparison of the post-operative recurrence pattern according to CD105 and CD31 expression.*

	CD105 expression			CD31 expression		
Type of recurrence	Low (n=71)	High (n=71)	*p*-Value	Low (n=71)	High (n=71)	*p*-Value
Hematogenous						
Negative	62	52	0.03	57	57	NS
Positive	9	19		14	14	
Lymph node						
Negative	57	51	NS	53	55	NS
Positive	14	20		18	16	

Table IV. *Univariate and multivariate analyses of prognostic factors in esophageal squamous cell carcinoma.*

Prognostic factors	Univariate P	Multivariate P	Hazard ratio (95%confidence interval)
pT (12/34)	<0.0001	0.002	0.382 (0.176-0.828)
pN (0/1)	<0.0001	0.445	0.760 (0.375-1.540)
ly (–/+)	<0.0001	0.023	0.315 (0.116-0.859)
v (–/+)	<0.0001	0.007	0.412 (0.216-0.784)
CD105 Low/ High	0.002	0.414	0.759 (0.455-1.383)

ly: Lymphatic invasion; v: venous invasion.

immunostain microvessels. Regarding the first issue, while there are various evaluation methods, de Jong *et al.* demonstrated that MVD counted in 4 or 5 fields at high power (×400) was the best method in predicting prognosis, compared to MVD in more or less fields (23). This group reported that the most reliable total area for estimating MVD was around 0.7 mm^2 and that the measurement of MVD by the hotspot method was preferred to the vascular localization method. Hence, in previous studies, that did not fulfil the above criteria, MVD would not be an independent prognosticator. Regarding the second issue, results appear to differ depending on the type of antibody used to evaluate MVD. Factor VIII antibody reacts with not only blood vessels but also with lymphatic tissue, platelets, megakaryocytes, mast cells, and background stroma (24, 25). CD31 has been considered more suitable for evaluation of MVD; however, it has also been reported to react with platelets, lymphatics and inflammatory cells (25, 26). Further, CD34 reacts with some mesenchymal cells (27, 28). Hence pan-endothelial markers such as factor VIII, CD31, and CD34 have low sensitivity and specificity for blood vessels and seem to be inadequate for an accurate measurement of MVD.

CD105 is a transmembrane glycoprotein and is a component of the transforming growth factor (TGF)-β receptor complex that is expressed on the surface of endothelial cells (29). By modulating TGF-β signalling, CD105 is involved in promoting the proliferation of endothelial cells (30). *In vivo*, CD105 knockout mice die at an early embryonic stage because blood vessels do not mature (31). CD105 is thought to be necessary for the early phase of remodelling new blood vessels, and, as a result, CD105 antibody binds preferentially to 'activated' endothelial cells (13, 14, 30, 32). Recent research revealed that promoter hypermethylation of CD105 appears to be a frequent event in the tumor of ESCC patients and exhibits a field defect with promising biomarker potential for the early detection of ESCC (33).

Based on these previous studies, we immunohisto-chemically investigated the correlation between CD105-MVD, CD31-MVD, and VEGF-A expression and clinicopathological factors including prognosis in ESCC. We investigated MVD in a total area of 0.72 mm^2 by the method of Weidner *et al.* (16). CD31-MVD was significantly associated with tumor length, depth of tumor invasion, lymph node metastasis, stage, lymphatic invasion, venous invasion and VEGF-A expression, but not with overall survival or disease-free survival. CD105-MVD showed a stronger correlation with all clinicopathological parameters than CD31-MVD. Furthermore, CD105-MVD but not CD 31-MVD was significantly correlated with overall survival and disease-free survival. In contrast with CD31-MVD, CD105-MVD predicted post-operative metastasis, particularly hematogenous metastasis. Taken together, these results suggest that CD105 expression can become an important marker of new blood vessels induced by ESCC as much as

CD31. In conclusion, we demonstrated that CD105 expression was related to malignant tumor properties, as well as prognosis, in ESCC. We also showed the usefulness of CD105 as a marker of angiogenesis. Although CD105 was not identified as an independent prognostic factor, further study of CD105 expression may be proven useful for determining malignant properties and clinical outcome in patients with ESCC.

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References

1 Hanahan D and Weinberg RA: The Hallmarks of Cancer. Cell *7*: 57-70, 2000.
2 Weidner N, Folkman J, Pozza F, Bevilacqua P, Allred EN, Moore DH, Meli S and Gasparini G: Tumor angiogenesis: a new significant and independent prognostic indicator in early-stage breast carcinoma. J Natl Cancer Inst *84*: 1875-1887, 1992.
3 Eisma RJ, Spiro JD and Kreutzer DL: Vascular Endothelial Growth Factor Expression in Head and Neck Squamous Cell Carcinoma. Am J Surg *174*: 513-517, 1997.
4 Cavallaro U and Christofori G: Molecular mechanisms of tumor angiogenesis and tumor progression. J Neurooncol *50*: 63-70, 2000.
5 Tanigawa N, Matsumura M, Amaya H, Kitaoka A, T Shimomatsuya, Lu C, Muraoka R and Tanaka T: Tumor vascularity correlates with the prognosis of patents with esophageal squamous cell carcinoma. Cancer *79*: 220-225, 1997.
6 De Jong JS, van Diest PJ and Baak JP: Hot spot microvessel density and the mitotic activity index are strong additional prognostic indicators in invasive breast cancer. Histopathology *36*: 306-312, 2000.
7 Matsuyama K, Chiba Y, Sasaki M, Tanaka H, Muraoka R and Tanigawa N: Tumor angiogenesis as a prognostic in operable non-small cell lung cancer. Ann Thorac Surg *65*: 1405-1409, 1998.
8 Logan-Collins JM, Lowy AM, Robinson-Smith TM, Kumar S, Sussman JJ, James LE and Ahmad SA: VEGF expression predicts survival in patients with peritoneal surface metastases from mucinous adenocarcinoma of the appendix and colon. Ann Surg Oncol *15*: 738-744, 2008.
9 Shih CH, Ozawa S, Ando N, Ueda M and Kitajima M: Vascular endothelial growth factor expression predicts outcome and lymph node metastasis in squamous cell carcinoma of the esophagus. Clin Cancer Res *6*: 1161-1168, 2000.
10 Gadducci A, Viacava P, Cosio S, Cecchetti D, Fanelli G, Fanucchi A, Teti G and Genazzani AR: Vascular endothelial growth factor (VEGF) expression in primary tumors and peritoneal metastasis from patients with advanced ovarian cancer. Anticancer Res *23*: 3001-3008, 2003.
11 Tanigawa N, Amaya H, Matsumura M and Shimomatsuya T: Correlation between expression of vascular endothelial growth factor and tumor vascularity, and patient outcome in human gastric carcinoma. J Clin oncol *15*: 826-832, 1997.
12 Zhang L, Zhou F, W Han, Shen B, Luo J, Shibuya M and He Y: VEGFR-3 ligand-binding and kinase activity are required for lymphangiogenesis but not for angiogenesis. Cell Res *20*: 1319-1331, 2010.
13 Yao Y, Pan Y, Chen J, Sun X, Qiu Y and Ding Y: Endoglin (CD105) expression in angiogenesis of primary hepatocellular carcinomas: analysis using tissue microarrays and comparison with CD34 and VEGF. Ann Clin Lab Sci *37*: 39-48, 2007.
14 Taskiran C, Erdem O, Onan A, Arisoy O, Acar A, Vural C, Erdem M, Ataoglu O and Guner H: The prognostic value of endoglin (CD105) expression in ovarian carcinoma, Int J Gynecol Cancer *16*: 1789-1793, 2006.
15 Sobin LH and Fleming ID: TNM Classification of malignant tumors, fifth edition. Cancer *80*: 1803-1804, 1997.
16 Weidner N, Semple JP, Welch WR and Folkman J: Tumor angiogenesis and metastasis-Correlation in invasive breast carcinoma. N Engl J Med *324*: 1-8, 1991.
17 Nakagawa S, Nishimaki T, Suzuki T, Kanda T, Kuwabara S and Hatakeyama K: Tumor angiogenesis as an independent prognostic factor after extended radical esophagectomy for invasive squamous cell carcinoma of the esophagus. Surgery *129*: 302-308, 2001.
18 Du JR, Jiang Y, Zhang YM and Fu H: Vascular endothelial growth factor and microvascular density in esophageal and gastric carcinomas. World J Gastroenterol *9*: 1604-1606, 2003.
19 Ding MX, Lin XQ, Fu XY, Zhang N and Li JC: Expression of vascular endothelial growth factor-C and angiogenesis in esophageal squamous cell carcinoma. World J Gastroenterol *12*: 4582-4585, 2006.
20 Saad RS, Lindener JL, Liu Y and Silverman JF: Lymphatic vessel density as prognostic marker in esophageal adenocarcinoma. Am J Clin Pathol *131*: 92-98, 2009.
21 Choi JY, Jang KT, Shim YM, Kim K, Ahn G, Lee KH, Choi Y, Choe YS and Kim BT: Prognostic significance of vascular endothelial growth factor expression and microvessel density in esophageal squamous cell carcinoma: comparison with position emission tomography. Ann Surg Oncol *13*: 1054-1062, 2006.
22 Igarashi M, Dhar DK, Kubota H, Yamamoto A, EI-Assal O and Nagasue N: The prognostic significance of microvessl density and thymidine phosphorylase expression in squamous cell carcinoma of the esophagus. Cancer *82*: 1225-1232, 1998.
23 de Jong JS, van Diest PJ and Baak JP: Hot spot microvessel density and the mitotic activity index are strong additional prognostic indicator in invasive breast cancer. Histopathlogy *36*: 306-312, 2000.
24 Parums DV, Cordell JL, Micklem K, Heryet AR, Gatter KC and Mason DY: JC70: a new monoclonal antibody that detects vascular endothelium associated antigen on routinely processed tissue sections. J Clin Pathol *43*: 752-757, 1990.
25 Miettinen M, Lindenmayer AE and Chaubal A: Endothelial cell markers CD31, CD34, and BNH9 antibody to H-andY-antigens-evaluation of their specificity and sensitivity in the diagnosis of vascular tumors and comparison with von Willerrand factor. Mod Pathol *7*: 82-90, 1994.
26 Giatromanolaki A, Sivridis E, Koukourakis MI, Georgoulias V, Gatter KC and Harris AL: Intratumoral angiogenesis: a new prognostic for stage I endometrial adenocarcinomas? Oncol Res *11*: 205-212, 1999.
27 Saad RS, Jasnosz KM and Tung MY: Silverman JF. Endoglin (CD105) expression in endometrial carcinoma. Int J Gynecol Pathol *22*: 248-253, 2003.

28 Lindenmayer AE and Miettinen M: Immunophenotypic features of uterine stromal cells. CD34 expression in endocervical stroma. Virchows Arch *426*: 457-460, 1995.
29 Cheifetz S, Bellon T, Cales C, Vera S, Bernabeu C, Massague J and Letarte M: Endoglin is a component of the transforming growth factor-beta receptor system in human endothelial cells. J Biol Chem *267*: 19027-19030, 1992.
30 Dallas NA, Samuel S, Xia L, Fan F, Gray MJ, Lim SJ and Ellis LM: Endoglin (CD105): a marker of tumor vasculature and potential target for therapy. Clin Cancer Res *14*: 1931-1937, 2008.
31 Bourdeau A, Faughnan ME and Letarte M: Endoglin-deficient mice, a unique model to study hereditary haemorrhagic telangiectasia. Trend Cardiovasc Med *10*: 279-285, 2000.
32 Li SL, Gao DL, Zhao ZH, Liu ZW, Zhao QM, Yu JX, Chen KS and Zhang YH: Correlation of matrix metalloproteinase suppressor genes RECK, VEGF, and CD105 with an angiogenesis and biological behavior in esophageal squamous cell carcinoma. World J Gastroenterol *13*: 6076-6081, 2007.
33 Jin Z, Zhao Z, Cheng Y, Dong M, Zhang X, Wang L, Fan X, Feng X, Mori Y and Meltzer SJ: Endoglin promoter hypermethylation identifies a field defect in human primary esophageal cancer. Cancer *119*: 3604-3609, 2013.

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