

Original

CD105 expression in oral capillary hemangiomas and cavernous hemangiomas

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Abstract: Capillary hemangioma (capillary lobular hemangioma) and cavernous hemangioma (venous malformation) are relatively common oral tumors/malformations and are characterized by increased numbers of normal and abnormal blood vessels. However, the causes of these lesions are not well understood. CD105 (endoglin) is predominantly expressed in proliferating blood endothelial cells (ECs). We analyzed expressions of CD105, CD34, von Willebrand factor, Ki-67, cyclooxygenase-2 (COX-2), and vascular endothelial growth factor (VEGF)-A in 31 capillary hemangiomas and 34 cavernous hemangiomas. Staining scores were calculated as the product of the proportion score and intensity score. Morphologically normal oral mucosa specimens ($n = 10$) were simultaneously evaluated as normal controls. As compared with cavernous hemangiomas and normal controls, capillary hemangiomas had higher staining scores for CD105, VEGF-A, and COX-2. The Ki-67 labeling index was significantly higher in capillary hemangiomas than in cavernous hemangiomas and normal controls ($P < 0.01$). These findings suggest that the biological characteristics of capillary and cavernous hemangiomas are quite different. The ECs of capillary hemangiomas actively proliferated and were generally regulated by VEGF-A. In contrast, the ECs of cavernous hemangiomas lacked proliferative

activity. These results suggest that angiogenesis and vasodilatation of pre-existing blood vessels are important in the development of capillary hemangioma and cavernous hemangioma, respectively.

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Keywords: CD105; capillary hemangioma; cavernous hemangioma; immunohistochemistry; angiogenesis.

Introduction

Hemangioma is one of the most common soft tissue tumors and represents 7% of all benign tumors (1). The head and neck are commonly affected, and the oral cavity is the most frequent site, accounting for 14% of all cases (1). Capillary and cavernous hemangiomas are the most frequent variants. The etiology of hemangiomas is controversial. Only some hemangiomas are true neoplasms; others are more likely to represent malformations (1). Capillary hemangioma is classified as a true tumor by the International Society for the Study of Vascular Anomalies (ISSVA) (2; http://issva.org/content.aspx?page_id=0&club_id=298433&module_id=152904) but is classified as a pyogenic granuloma (lobular capillary hemangioma) in the World Health Organization (WHO) classification of skin tumors (3). Chiu et al. reported that capillary hemangiomas exhibit clonal X-chromosome inactivation and should thus be considered true neoplasms (4). In contrast, cavernous hemangioma is considered a venous malformation by the ISSVA and WHO (2,3).

Several markers are used to identify blood endothelial cells (ECs), including CD34, von Willebrand factor

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(vWF), and CD105 (endoglin). CD34 is a 110-kDa transmembrane glycoprotein with high specificity in blood ECs, lymphohematopoietic stem and progenitor cells, and leukemic cells (5). Factor VIII/vWF complex is a large glycoprotein with a multimeric structure and a wide range of masses, from 500 to more than 10,000 kDa. vWF is widely used as a blood endothelial cell marker. It mediates platelet adhesion and thrombus formation at sites of vascular injury and serves as a carrier for factor VIII in plasma, protecting the circulating coagulation enzyme from proteolytic degradation (6). The protein is present in plasma, platelets, megakaryocytes, and endothelial cells of the vessel wall (7). CD105 is a 180-kDa homodimeric transmembrane protein that acts as a component of the transforming growth factor- β receptor complex and binds TGFB1 and TGFB3 with high affinity (8,9). It is expressed by actively proliferating ECs and is important in angiogenesis, vascular homeostasis, and cardiovascular development (10). Vascular endothelial growth factor A (VEGF-A) is a key regulator of angiogenesis and enhances blood EC proliferation, migration, and differentiation (11). It is expressed in a variety of tissue and cells, including the endometrial gland, salivary gland, and mucosa of the gastrointestinal tract. In addition, VEGF-A is identified in plasma cells, fibroblasts, lymphocytes, plasma in the lumen of some blood vessels, focal areas of the interstitium (12), and in hypoxic conditions (11). VEGF-A is believed to be a causative cytokine in infantile hemangioma (13). Cyclooxygenase-2 (COX-2) is an inducible enzyme involved in metabolic conversion of arachidonic acid to prostaglandins and is important in angiogenesis (14,15).

We investigated the biological characteristics of capillary and cavernous hemangiomas by determining expression levels of the endothelial cell markers CD34, vWF, and CD105 and angiogenic factors VEGF-A and COX-2.

Materials and Methods

Patients and samples

Thirty-one cases of capillary hemangioma and 34 cases of cavernous hemangioma diagnosed between 2004 and 2010 were extracted from the pathology records of Nihon University School of Dentistry Dental Hospital. Cases with findings of papillary endothelial hyperplasia were excluded from the analysis. Nonpathologic mucosa specimens were used as normal controls. All case records were reviewed by certified oral pathologists (NM, KK). Among the 31 cases of capillary hemangioma, 18 (58.1%) of the patients were male and 13 (41.9%) were female; mean patient age was 56.7 ± 16.9 years (range,

Table 1 Comparison of clinical features of oral hemangioma cases

	Capillary hemangioma (n = 31)	Cavernous hemangioma (n = 34)
Age (years, mean \pm SD)	56.7 \pm 16.9	59.1 \pm 17.8
Gender		
Male	18 (58.1%)	13 (38.2%)
Female	13 (41.9%)	21 (61.8%)
Tumor site		
Buccal mucosa	14 (45.2%)	14 (41.2%)
Tongue	11 (35.5%)	7 (20.6%)
Lip	3 (9.7%)	12 (35.3%)
Gingiva	2 (6.5%)	1 (2.9%)
Palate	1 (3.2%)	0 (0.0%)

7-83 years). Most lesions were located on buccal mucosa (45.2%), followed by the tongue (35.5%), lip (9.7%), gingiva (6.5%), and palate (3.2%). Among the 34 cases of cavernous hemangioma, 13 (38.2%) of the patients were male and 21 (61.8%) were female; mean patient age was 59.1 ± 17.8 years (range, 19-83 years). Most lesions were located on buccal mucosa (41.2%), followed by the lip (35.3%), tongue (20.6%), and gingiva (2.9%). The case profiles are summarized in Table 1. The normal controls were 10 samples of oral mucosa without inflammation or neoplasm. All specimens obtained from surgically excised tissues were routinely fixed in 10% neutral buffered formalin and embedded in paraffin. Serial sections (thickness, 4 μ m) were cut from each paraffin block and used for hematoxylin and eosin staining and immunohistochemical evaluation. This study was approved by the Ethical Committee of Nihon University School of Dentistry (2007-25).

Immunohistochemistry

The sections obtained from paraffin-embedded tissues were incubated with anti-human CD34, vWF, CD105, Ki-67, VEGF-A, and COX-2 antibodies. Briefly, after deparaffinization and rehydration, the sections were subjected to epitope retrieval by protease digestion or heat treatment. The sections were incubated with primary antibodies for 2 h at room temperature, after which endogenous peroxidase activity was quenched by incubating them with 3% hydrogen peroxide for 5 min at room temperature. The slides were then incubated with horseradish peroxidase-labeled secondary antibody (Envision, K1491, Dako, Glostrup, Denmark) for 45 min at room temperature. All slides were visualized with 3,3'-diaminobenzidine (Sigma-Aldrich, St. Louis, MO, USA). Sections were counterstained with Mayer's

Table 2 Primary antibodies used in the present study

Antibody	Clone	Dilution	Pretreatment	Manufacturer
CD34	QBEnd	1/100	CB, Heat	Dako, Glostrup, Denmark
vWF	*	1/200	Trypsin	Invitrogen, Waltham, MA, USA
CD105	SN6h	1/10	Proteinase K	Dako, Glostrup, Denmark
COX-2	*	1/100	CB, Heat	Abcam, Cambridge, UK
VEGF-A	*	1/100	None	Santa Cruz Biotechnology, Dallas, TX, USA
Ki-67	MIB-1	1/150	CB, AC	Dako, Glostrup, Denmark

vWF, von Willebrand factor; COX-2, cyclooxygenase-2; VEGF-A, vascular endothelial growth factor A; CB, citrate buffer; Heat, incubation at 98°C for 20 min; AC, autoclave

*rabbit polyclonal antibody

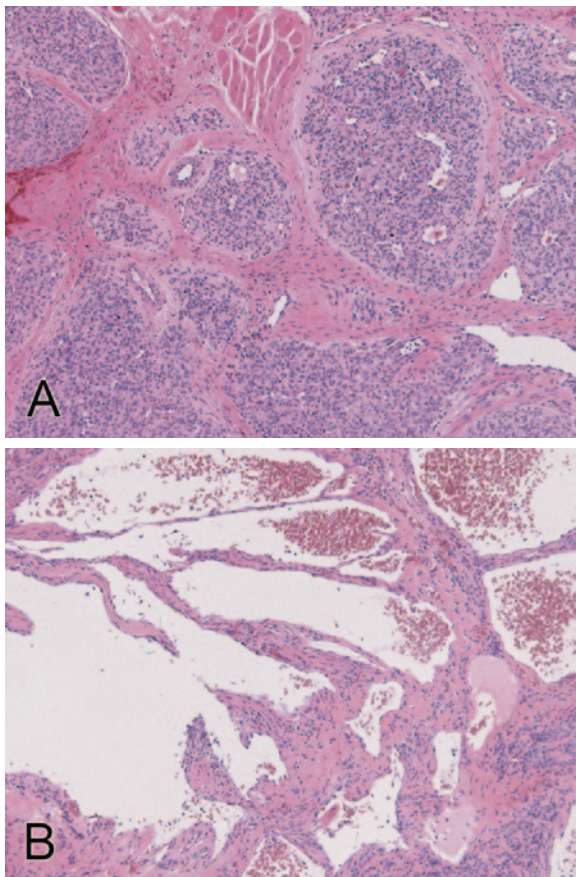


Fig. 1 Histologic findings of capillary and cavernous hemangiomas. **(A)** Capillary hemangioma showing lobular proliferation of numerous blood vessels, which occasionally contain inflammatory cells. **(B)** Cavernous hemangioma showing dilated blood spaces, with vessels separated by focally hyalinized fibrous connective tissue. (1A, 1B; $\times 10$ objective lens)

hematoxylin. The antibodies and staining conditions are summarized in Table 2. Appropriate positive and negative controls were used.

Evaluation of staining score

The immunohistochemical staining pattern was analyzed

using light microscopy. Expression levels of CD34, vWF, and CD105 in blood ECs were reported as staining scores. Staining score (0-9) was calculated using a modification of the method of Wakulich (16) and expressed as the product of the staining proportion score (0-3) and staining intensity score (0-3). The staining proportion score of 0-3 was determined as follows: 0, 0%; 1, 0%-20%; 2, 20%-50%; and 3, 50%-100% positive rate of blood ECs. Instead of number of positive ECs, the number of VEGF-A- and COX-2-positive cells in 10 high power fields were counted. The proportion score of 0-3 was determined as follows: 0, absence; 1, lower one-third of the number; 2, median one-third of the number; and 3, higher one-third of the number. The staining intensity score of 0-3 was determined as follows: 0, absence of staining; 1, weak staining; 2, moderate staining; and 3, generalized intense staining. The Ki-67 labeling index was defined as the average percentage of Ki-67-positive nuclei in ECs within five different blood vessels.

Statistical analysis

Staining scores and Ki-67 labeling indices were statistically compared using non-repeated measures ANOVA with Student-Newman-Kuels test correction. A *P*-value of <0.05 was considered to indicate statistical significance.

Results

Capillary hemangiomas showed lobular proliferation of numerous blood vessels, which occasionally contained inflammatory cells. In contrast, cavernous hemangiomas showed dilated blood spaces, with vessels separated by focally hyalinized fibrous connective tissue (Fig. 1). Expression levels of the endothelial markers CD34, vWF, and CD105 and angiogenic factors VEGF-A and COX-2 were examined by a semiquantitative method using staining score (0-9), ie, the product of proportion score (0-3) and intensity score (0-3). Proportion, intensity, and

Table 3 Staining scores, proportion scores, and intensity scores for capillary hemangiomas, cavernous hemangiomas, and normal controls

		Normal control (n = 10)							Capillary hemangioma (n = 31)							Cavernous hemangioma (n = 34)						
CD34	SS	0	1	2	3	4	6	9	0	1	2	3	4	6	9	0	1	2	3	4	6	9
	n	0	0	0	0	0	1	9	0	0	4	1	0	11	15	8	8	12	3	2	1	0
		(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(12.9%)	(6.5%)	(0.0%)	(32.3%)	(48.4%)	(23.5%)	(23.5%)	(35.3%)	(8.8%)	(5.9%)	(2.9%)	(0.0%)
PS		0		1		2		3	0		1		2		3	0		1		2		3
	n	0		0		0		10	0		5		8		18	8		8		14		4
		(0.0%)		(0.0%)		(0.0%)		(100.0%)	(0.0%)		(16.1%)		(25.8%)		(58.1%)	(23.5%)		(23.5%)		(41.2%)		(11.8%)
IS		0		1		2		3	0		1		2		3	0		1		2		3
	n	0		0		1		9	0		0		7		24	8		23		3		0
		(0.0%)		(0.0%)		(10.0%)		(90.0%)	(0.0%)		(0.0%)		(22.6%)		(77.4%)	(23.5%)		(67.6%)		(8.8%)		(0.0%)
CD105	SS	0	1	2	3	4	6	9	0	1	2	3	4	6	9	0	1	2	3	4	6	9
	n	7	1	2	0	0	0	0	2	0	0	1	2	10	16	22	7	3	0	1	1	0
		(70.0%)	(10.0%)	(20.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(6.5%)	(0.0%)	(0.0%)	(3.2%)	(6.5%)	(32.3%)	(51.6%)	(6.5%)	(20.6%)	(8.8%)	(0.0%)	(2.9%)	(2.9%)	(0.0%)
PS		0		1		2		3	0		1		2		3	0		1		2		3
	n	7		2		1		0	1		1		3		26	23		8		3		0
		(70.0%)		(20.0%)		(10.0%)		(0.0%)	(3.2%)		(3.2%)		(9.7%)		(83.9%)	(67.6%)		(23.5%)		(8.8%)		(0.0%)
IS		0		1		2		3	0		1		2		3	0		1		2		3
	n	7		2		1		0	1		1		11		18	22		8		3		1
		(70.0%)		(20.0%)		(10.0%)		(0.0%)	(3.2%)		(3.2%)		(35.5%)		(58.1%)	(64.7%)		(23.5%)		(8.8%)		(2.9%)
vWF	SS	0	1	2	3	4	6	9	0	1	2	3	4	6	9	0	1	2	3	4	6	9
	n	0	0	0	0	3	5	2	7	3	4	2	1	10	4	10	10	7	2	3	2	0
		(0.0%)	(0.0%)	(0.0%)	(0.0%)	(30.0%)	(50.0%)	(20.0%)	(22.6%)	(9.7%)	(12.9%)	(6.5%)	(3.2%)	(32.3%)	(12.9%)	(29.4%)	(29.4%)	(20.6%)	(5.9%)	(8.8%)	(5.9%)	(0.0%)
PS		0		1		2		3	0		1		2		3	0		1		2		3
	n	0		0		6		4	5		5		5		16	10		16		7		1
		(0.0%)		(0.0%)		(60.0%)		(40.0%)	(16.1%)		(16.1%)		(16.1%)		(51.6%)	(29.4%)		(47.1%)		(20.6%)		(2.9%)
IS		0		1		2		3	0		1		2		3	0		1		2		3
	n	0		0		5		5	7		7		11		6	10		13		8		3
		(0.0%)		(0.0%)		(50.0%)		(50.0%)	(22.6%)		(22.6%)		(35.5%)		(19.4%)	(29.4%)		(38.2%)		(23.5%)		(8.8%)
VEGF-A	SS	0	1	2	3	4	6	9	0	1	2	3	4	6	9	0	1	2	3	4	6	9
	n	0	0	5	0	3	2	0	0	0	4	1	2	7	17	1	0	7	12	9	5	0
		(0.0%)	(0.0%)	(50.0%)	(0.0%)	(30.0%)	(20.0%)	(0.0%)	(0.0%)	(0.0%)	(12.9%)	(3.2%)	(6.5%)	(22.6%)	(54.8%)	(2.9%)	(0.0%)	(20.6%)	(35.3%)	(26.5%)	(14.7%)	(0.0%)
PS		0		1		2		3	0		1		2		3	0		1		2		3
	n	0		4		5		1	0		2		7		22	1		12		18		3
		(0.0%)		(40.0%)		(50.0%)		(10.0%)	(0.0%)		(6.5%)		(22.6%)		(71.0%)	(2.9%)		(35.3%)		(52.9%)		(8.8%)
IS		0		1		2		3	0		1		2		3	0		1		2		3
	n	0		1		8		1	0		3		8		20	1		7		12		14
		(0.0%)		(10.0%)		(80.0%)		(10.0%)	(0.0%)		(9.7%)		(25.8%)		(64.5%)	(2.9%)		(20.6%)		(38.2%)		(41.2%)
COX-2	SS	0	1	2	3	4	6	9	0	1	2	3	4	6	9	0	1	2	3	4	6	9
	n	0	1	4	0	4	1	0	2	1	5	8	2	13	0	11	5	10	6	2	0	0
		(0.0%)	(10.0%)	(40.0%)	(0.0%)	(40.0%)	(10.0%)	(0.0%)	(6.5%)	(3.2%)	(16.1%)	(25.8%)	(6.5%)	(41.9%)	(0.0%)	(32.4%)	(14.7%)	(29.4%)	(17.6%)	(5.9%)	(0.0%)	(0.0%)
PS		0		1		2		3	0		1		2		3	0		1		2		3
	n	0		1		8		1	2		1		9		19	10		22		2		0
		(0.0%)		(10.0%)		(80.0%)		(10.0%)	(6.5%)		(3.2)		(29.0%)		(61.3%)	(0.0%)		(64.7%)		(5.9%)		(2.9%)
IS		0		1		2		3	0		1		2		3	0		1		2		3
	n	0		5		5		0	2		14		13		2	11		5		12		6
		(0.0%)		(50.0%)		(50.0%)		(0.0%)	(6.5%)		(45.2%)		(41.9%)		(6.5%)	(32.4%)		(14.7%)		(35.3%)		(17.6%)

SS, staining score; PS, proportion score; IS, intensity score

staining scores are summarized in Table 3 and Fig. 2.

CD34

Strong immunohistochemical staining was seen in the submucosa of normal control capillaries (Fig. 2A). Capillary hemangiomas exhibited moderate-to-strong CD34 staining of blood ECs (Fig. 2B). The staining intensity of CD34 was lower in cavernous hemangiomas (Fig. 2C); 7 (20.6%) of the 34 cavernous hemangioma cases analyzed were completely negative for CD34 protein expression. CD34 staining score was significantly higher in normal

control samples (8.7 ± 0.9) than in capillary hemangiomas (6.8 ± 2.5 , $P < 0.01$) and cavernous hemangiomas (1.6 ± 1.4 , $P < 0.01$) (Fig. 3A)

vWF

Strong staining intensity was seen in normal controls (Fig. 2D). Capillary hemangiomas exhibited moderate staining intensity (Fig. 2E), but cavernous hemangiomas exhibited weak expression (Fig. 2F). Staining score was significantly higher in normal controls (6.0 ± 1.8) than in capillary hemangiomas (3.8 ± 3.1 , $P < 0.05$) and

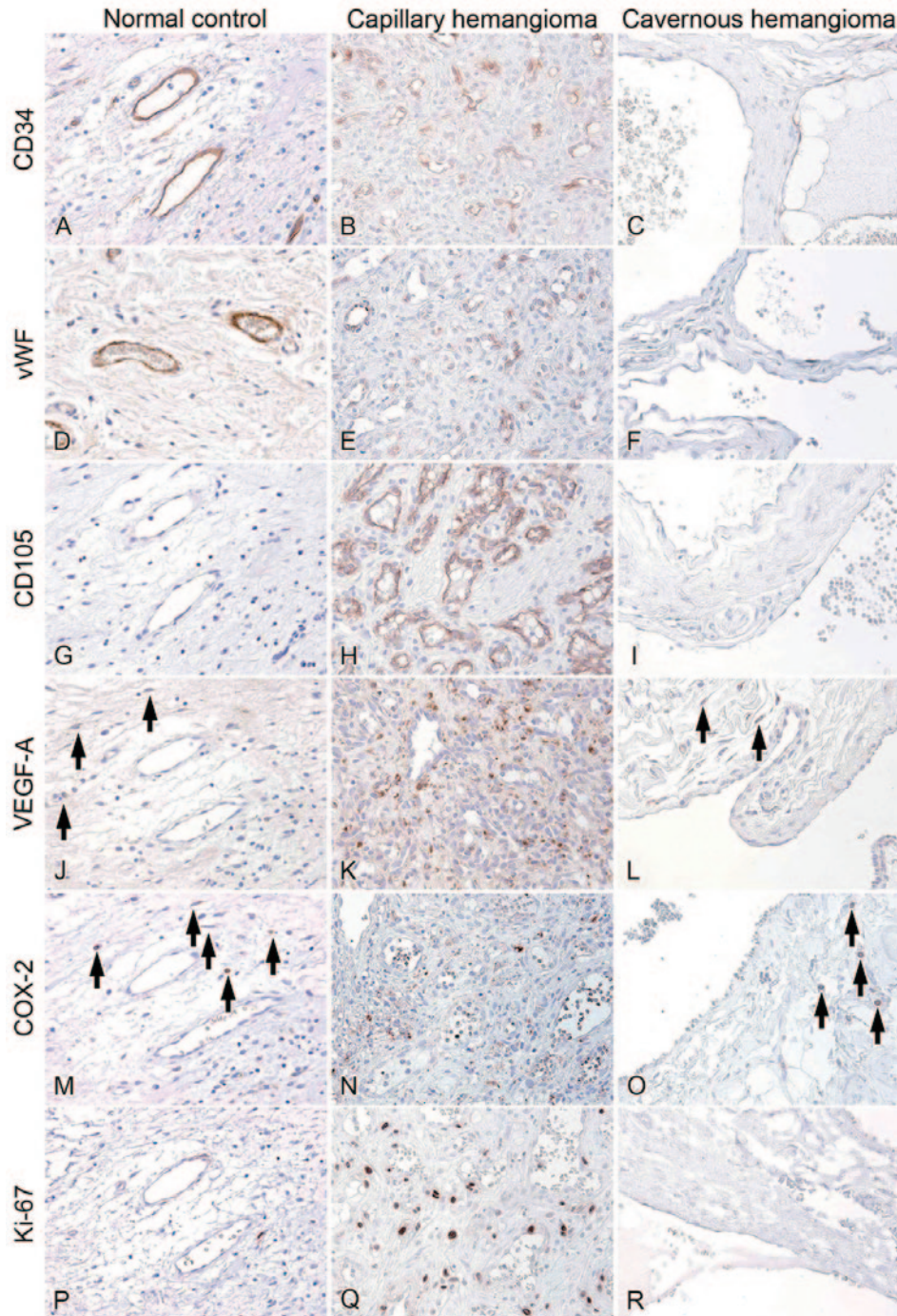


Fig. 2 Immunohistochemical findings for endothelial cell markers and angiogenic factors. Expressions of CD34, vWF, CD105, VEGF-A, COX-2, and Ki-67 were investigated in normal controls, capillary hemangiomas, and cavernous hemangiomas. (A) Normal controls showed intense expression of CD34 (SS = 9, PS = 3, IS = 3); CD34 expression gradually decreased in (B) capillary hemangiomas (SS = 6, PS = 3, IS = 2) and (C) cavernous hemangiomas (SS = 1, PS = 1, IS = 1). (D) Expression of vWF was higher in normal controls (SS = 9, PS = 3, IS = 3) than in (E) capillary hemangiomas (SS = 4, PS = 2, IS = 2) and (F) cavernous hemangiomas (SS = 0, PS = 0, IS = 0). (G) CD105 expression was rarely seen in normal controls (SS = 0, PS = 0, IS = 0); however, (H) capillary hemangiomas showed intense expression (SS = 9, PS = 3, IS = 3). (I) CD105 expression was very similar in cavernous hemangiomas and normal controls (SS = 0, PS = 0, IS = 0). (J) Few VEGF-A-positive cells were identified in normal controls (arrows, positive cells; SS = 1, PS = 1, IS = 1); however, (K) VEGF-A-positive cells were dispersed in the stromal tissue in capillary hemangiomas (SS = 9, PS = 3, IS = 3). (L) VEGF-A-positive cells were occasionally found in cavernous hemangiomas (arrows, positive cells; SS = 4, PS = 2, IS = 2). (M) COX-2-positive cells were rarely seen in the stroma of normal controls (arrows, positive cells; SS = 2, PS = 2, IS = 1). (N) COX-2-positive cells were frequently found in capillary hemangiomas (SS = 4, PS = 2, IS = 2) and (O) occasionally observed in cavernous hemangiomas (arrows, positive cells; SS = 2, PS = 2, IS = 1). (P) Ki-67 expression was negative in normal controls. In contrast, (Q) blood ECs often expressed Ki-67 in capillary hemangiomas. (R) A few positive cells were seen in cavernous hemangiomas. (2A-2R; $\times 40$ objective lens)

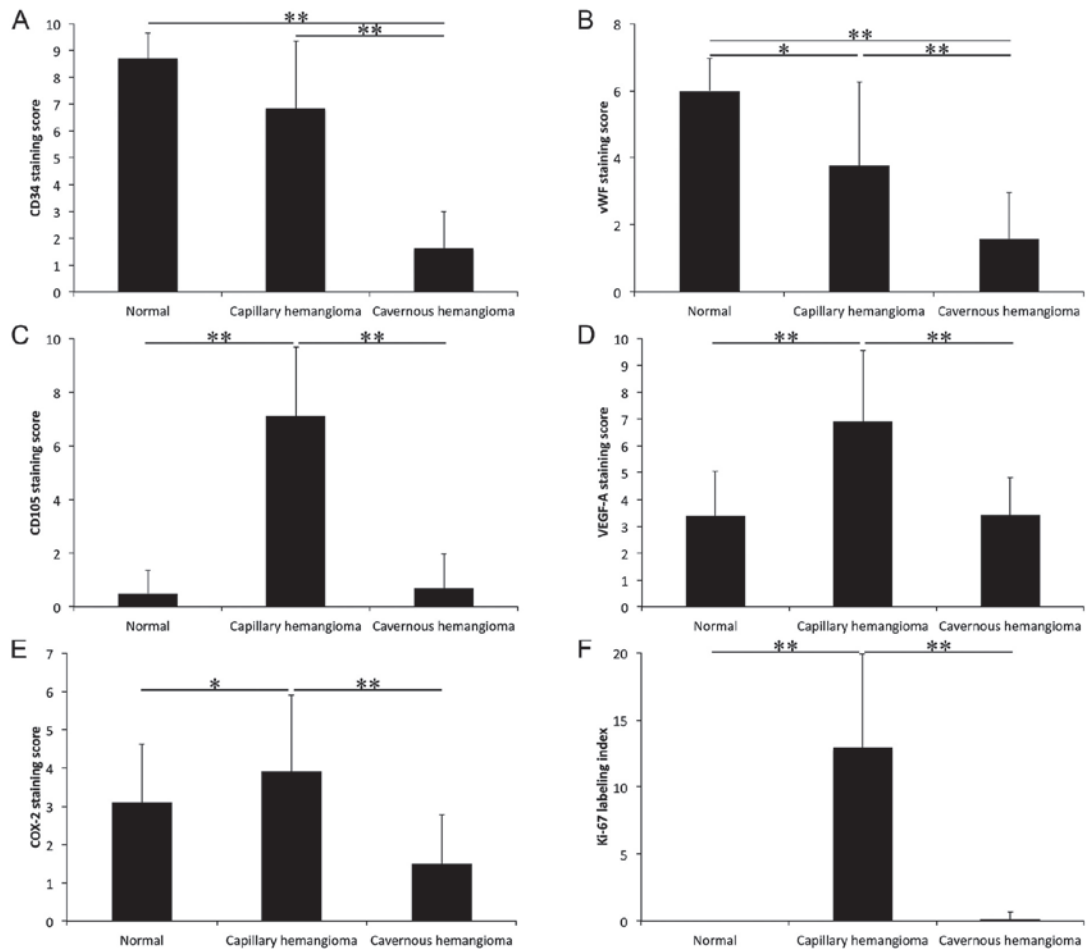


Fig. 3 Staining scores for blood endothelial markers, angiogenic factors, and Ki-67 labeling index.

To determine expression levels of the endothelial markers CD34, vWF, and CD105 and angiogenic factors VEGF-A and COX-2, staining scores were obtained by calculating the product of the proportion score (0-3) and intensity score (0-3). The Ki-67 labeling index was calculated as the percentage of 10 high-power field measurements. (* $P < 0.05$, ** $P < 0.01$)

cavernous hemangiomas (1.6 ± 1.7 , $P < 0.01$). Moreover, staining score significantly differed ($P < 0.01$) between the two types of hemangiomas (Fig. 3B).

CD105

CD105 expression was seen in only a few vessels in the submucosa of normal control (Fig. 2G). All capillary hemangioma cases showed moderate-to-strong CD105 staining of blood ECs (Fig. 2H). Nine (26.5%) of the 34 cavernous hemangioma cases analyzed were completely negative for CD105 (Fig. 2I). The staining score for CD105 was significantly higher in capillary hemangiomas (7.1 ± 2.6) than in normal controls (0.5 ± 0.8) and cavernous hemangiomas (0.7 ± 1.3) ($P < 0.01$ for both comparisons, Fig. 3C).

VEGF-A

Positive staining for VEGF-A was identified in stromal

fibroblasts and macrophages. A small number of VEGF-A-positive cells were seen in normal controls (Fig. 2J). Capillary hemangiomas showed moderate-to-strong staining (Fig. 2K). VEGF-A staining intensity was lower in cavernous hemangioma cases (Fig. 2L). Staining score was significantly higher for capillary hemangiomas (3.4 ± 1.6) than for normal controls (6.9 ± 2.7 , $P < 0.01$) and cavernous hemangiomas (3.4 ± 1.4 , $P < 0.01$) (Fig. 3D).

COX-2

COX-2-positive staining was observed in stromal fibroblasts and macrophages. The number of COX-2-positive cells was lower in normal controls (Fig. 2M). All capillary hemangiomas showed weak-to-moderate staining (Fig. 2N). Cavernous hemangiomas had the fewest COX-2-positive cells (Fig. 2O). Staining scores were significantly higher for capillary hemangiomas (3.9 ± 2.0) than for normal controls (3.1 ± 1.5 , $P < 0.05$) and

cavernous hemangiomas (1.5 ± 1.3 , $P < 0.01$) (Fig. 3E)

Ki-67

Ki-67-positive ECs were rare in normal control cases (Fig. 2P); however, ECs were frequently positive in capillary hemangiomas (Fig. 2Q). Cavernous hemangioma cases had a few Ki-67-positive ECs (Fig. 2R). The Ki-67 labeling index was significantly higher for capillary hemangiomas ($12.9 \pm 7.0\%$, $P < 0.01$) than for cavernous hemangiomas ($0.2 \pm 0.6\%$, $P < 0.01$) and normal controls ($0.0 \pm 0.0\%$, $P < 0.01$) (Fig. 3F). Positive cell nuclei were more swollen than negative cell nuclei.

Discussion

We determined expressions of three endothelial cell markers (CD34, vWF, and CD105) and two angiogenic factors (VEGF-A and COX-2) in capillary and cavernous hemangiomas in order to investigate the biological characteristics of these hemangiomas. Staining scores for CD105, VEGF-A, and Ki-67 labeling indices were significantly higher for capillary hemangiomas than for cavernous hemangiomas or normal controls.

CD34 staining score was significantly higher for normal mucosa than for cavernous hemangiomas. CD34-positive score was lower for capillary hemangiomas than for normal mucosa; however, the difference was not significant. Some previous studies showed that CD34 expression was positive in capillary hemangiomas (17-22). Wood et al. found that although CD34 was highly expressed in pre-ECs and in vessels formed by vasculogenesis and angiogenesis, expression was low in vessels formed by coalescence (23). Thus, capillary hemangiomas are likely formed by vasculogenesis or angiogenesis.

We observed strong expression of CD105 in all capillary hemangiomas. This finding confirms previous results (24,25). In the present study, CD105 expression was very weak in cavernous hemangiomas and normal tissues. Soares et al. hypothesized that CD105 could be used to distinguish newly forming vessels from pre-existing vessels (24). CD105 is a co-receptor of TGF- β 1 and - β 3. TGF- β is a widely expressed cytokine that regulates cellular responses in ECs and has been implicated in vascular malformations (26). Inhibition of CD105 activity in human umbilical vein cells suppressed VEGF-induced EC migration and angiogenesis (27). Thus, CD105 is required for efficient VEGF-induced angiogenesis. CD105 was rarely expressed in ECs of normal controls and cavernous hemangiomas, which suggests that it mediates EC proliferation and migration in capillary hemangiomas but not in cavernous heman-

giomas.

Expression of vWF was higher in normal controls than in capillary hemangiomas and cavernous hemangiomas. These findings confirm those of earlier studies (28). In our study, staining scores for vWF expression were almost equal for capillary and cavernous hemangiomas. Saul et al. showed that capillary and cavernous hemangiomas exhibited strong and weak-to-moderate positivity, respectively (17). Expression of vWF is lower in capillary hemangiomas and cavernous hemangiomas than in normal tissue.

The Ki-67 labeling index was greater in ECs of capillary hemangiomas than in those of cavernous hemangiomas and normal controls. These findings are compatible with those of a previous study (29). Similar results were seen for the CD105 staining index. Consistent with our results, Miller et al. found a correlation between CD105 and Ki-67 expression in blood vessels in lung tumors (30).

VEGF-A-positive cells are present in stromal fibroblasts and inflammatory cells. VEGF-A staining score was higher in capillary hemangiomas than in cavernous hemangiomas and normal controls, which confirms the findings of previous studies (19,31) and suggests that VEGF-A overexpression causes proliferation of blood vessels in capillary hemangiomas. Yuan et al. suggested that capillary hemangiomas are caused by an imbalance between angiogenesis enhancers and inhibitors (31). Bragado et al. hypothesized that early VEGF-A expression by endothelial cell precursors induces endothelial cell proliferation and that this phenomenon might lead to involution of capillary hemangiomas (19). At present, no data are available to identify the mechanism of VEGF-A expression in capillary hemangiomas.

COX-2-positive cells are found in stromal fibroblasts and macrophages. COX-2 staining score was high in cavernous and capillary hemangiomas; however, no significant difference was observed in COX-2 expression in capillary hemangiomas and normal controls. COX-2 is important in VEGF-induced angiogenesis via the p38 and JNK kinase activation pathways (32). Hence, the high staining scores for COX-2 and VEGF-A might reflect the biological characteristics of capillary hemangioma.

In summary, the biological characteristics of the investigated hemangiomas differed. The blood ECs of capillary hemangiomas have an active proliferative capacity, which is reflected in the significantly elevated expression of CD105 and Ki-67. VEGF-A- and COX-2-positive cells may be involved in the proliferative activity of ECs. In contrast, the ECs of cavernous hemangiomas had low levels of CD105 and Ki-67. These results suggest that

dilatation of pre-existing blood vessels contributes to the development of cavernous hemangiomas. However, the mechanism responsible for formation of dilated vessels remains unclear and should be investigated in future research.

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