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ORIGINAL ARTICLE

VEGF-C and VEGFR-3 in a series of lymphangiomas: Is superficial lymphangioma a true lymphangioma?

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Abstract Lymphangiomas are commonly regarded as vascular malformations during embryonic development rather than as true neoplasms. VEGF-C and VEGFR-3 are known to be active in the formation of lymphangiomas. However, the significance of the disorders seems to be obscured by confusing different entities. In 114 lymphangiomas, we investigated the clinicopathological features and the expression of VEGF-C and VEGFR-3. The age of patients with lymphangioma circumscriptum or intraabdominal lymphangioma was significantly higher than in patients with cavernous lymphangioma and in patients with cystic hygroma. In cavernous lymphangioma, the age of female patients was significantly higher than in male patients. Five adult cystic hygromas were identified. VEGF-C was detected in 21 of 58 (36%) cavernous lymphangiomas, ten of 28 (36%) cystic hygromas, 0 of 12 (0%) lymphangioma circumscriptum, and four of ten (40%) intraabdominal lymphangiomas. VEGFR-3 was detected in 43 of 58 (72%) cavernous lymphangiomas, 20 of 28 (71%) cystic hygromas, six of 12 (50%) lymphangiomas circumscriptum, and seven of ten (70%) intraabdominal lymphangiomas. VEGF-C was absent from superficial lymphangiomas associated with cavernous lymphangiomas. In typical cases of cavernous lymphangioma, VEGF-C was strongly expressed, suggesting that these cases possessed proliferative activity. In cystic hygroma and intraabdominal lymphangioma, VEGF-C was limited in its distribution. Superficial lymphangiomas more likely represent from peripheral lymphatic dilatation rather than due to growth factor.

Keywords Cystic hygroma · Head and neck neoplasm · Lymphangioma · Skin neoplasm · Soft tissue neoplasm · Vascular neoplasm · VEGF-C · VEGFR-3

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Introduction

Lymphangiomas are benign tumor-like lesions of lymphatics. Whereas hemangiomas have distinctive proliferative and involutional growth phases that distinguish them from vascular malformation [1–3], lymphangiomas are generally considered to be either hamartomas or the linkage between a malformation and a neoplasm. Some lesions exhibit progressive growth and infiltration, leaving open the question of whether they are true neoplasms or hamartomas [4].

Lymphangiomas are often recognized at birth or during infancy, but may also appear spontaneously in adolescence or later life. Traditionally, they have been divided into several types that differ in clinical and histological characteristics. Cavernous lymphangioma is the most common type and is usually present at birth or in infancy. Cystic hygroma is typically seen in the neck at birth. Intraabdominal lymphangiomas are reported to occur most

commonly in the mesentery, followed by the omentum, mesocolon, and retroperitoneum. Lymphangioma circumscriptum is a superficial lesion commonly involving the axilla, adjacent chest wall, oral cavity, and genitals. It may occur at any age, but usually beyond infancy. This form can be primary and secondary. The secondary form occurs in association with lymph stasis following trauma, surgery, radiation, infection, or chronic immobility [5–7].

Vascular endothelial growth factor-C (VEGF-C), in association with its major receptor, VEGF receptor-3 (VEGFR-3; also known as flt4), plays a role in the regulation of lymphatic system development [8, 9]. During late embryogenesis, VEGFR-3 is exclusively expressed in lymphatic endothelial cells [10], lining a network of vessels that drain interstitial fluid and cells from tissues. Excessive production of VEGF-C is associated with increased lymphangiogenesis, as reported in transgenic mice [11]. It has also been proposed that in solid tumors, VEGF-C secreted by cancer cells induces lymphangiogenesis, possibly leading to an increase in tumor metastasis via the lymphatic system [12]. In a study on a small series of lymphangiomas, co-expression of VEGF-C and VEGFR-3 was found in lymphatic endothelial cells, suggesting a role for the positive regulation of lymphangiogenesis [13]. More recently, experimental evidence has been provided for up-regulation of VEGFR-3 in lymphatic endothelial cells from lymphangiomas [14, 15]. We are now reporting the expression patterns of VEGF-C and VEGFR-3 in a large series of lymphangiomas.

Materials and methods

Sample collection

One hundred fourteen cases of lymphangiomas were selected from the 1981–2001 archival files of Kyushu University. All the histological slides were reviewed and reclassified. A case in this series has been previously reported [16]. Biopsy specimens from three cases of intestinal lymphangiectasia were also included in the study.

Immunohistochemistry

Formalin-fixed, paraffin-embedded sections (4 μm thick) were processed for immunohistochemistry using a standard streptavidin–biotin–peroxidase method. Primary antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Slides for VEGF-C were digested in 0.1% trypsin for 30 min, and for VEGFR-3, antigen was retrieved by microwave heating. Sections were incubated with primary antibody (1:200) overnight at 4°C. After the development of antibody-bridge labeling, sections were reacted with diaminobenzidine and counterstained. The number of immunoreactive cells was estimated semi-quantitatively as follows: grade 3+, >50% positive cells; grade 2+, 25–50% positive cells; grade 1+, <25% positive cells; −, negative staining [17].

Statistical analysis

Categorical variables were examined by Fisher's exact test. Age of patients was compared with Welch's *t* test and with Welch's one-way analysis of variance (ANOVA) if comparisons involved more than two groups because normal distributions were not obtained. Welch's ANOVA was followed by *post hoc* Holm–Bonferroni test for multiple comparisons. Twenty precent trimmed mean of age and standard error (SE) were also calculated [18]. Immunohistochemical scores were compared with Mann–Whitney *U* test. Spearman's rank correlation was performed to determine whether the immunohistochemical scores were correlated with age or with each other. "Cystic variant" of cavernous lymphangioma was excluded when Fisher's exact test and Spearman's procedure were applied. All data were analyzed by the statistical package R: a language and environment for statistical computing (http://www.R-project.org/). A probability value less than 0.05 was considered significant. Two-tailed *P* values were quoted throughout.

Results

Clinicopathological findings

The 114 patients whose samples were included in the study ranged in age from birth to 79 years (mean, 19.3 years;

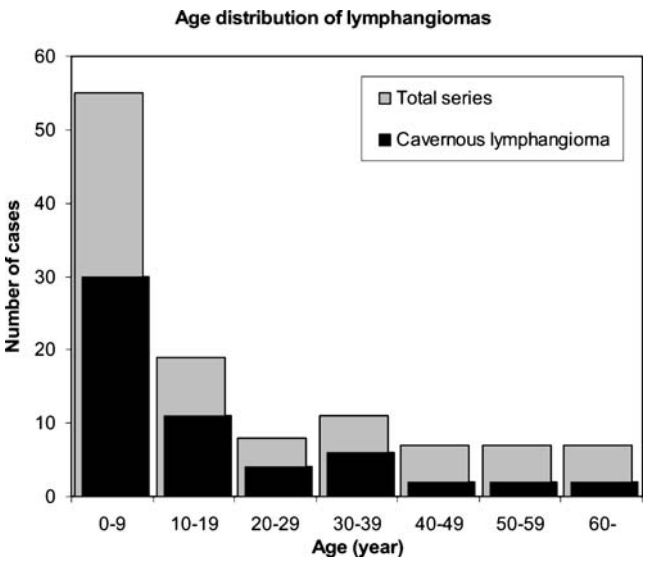

Fig. 1 Age distribution of the total series (n=114) and cavernous lymphangioma (n=58)

Table 1 Comparison of baseline clinical characteristics

		Age (mean, years)	Sex (M:F)
Cavernous lymphangioma	(*n*=58)	15.8	29:29
Cystic hygroma	(*n*=28)	8.5	14:14
Lymphangioma circumscriptum	(*n*=12)	37.1	4:8
Intra-abdominal lymphangioma	(*n*=10)	38.8	5:5
Total series	(*n*=114)	19.3	54:60
P value		*P*=0.0002	*P*=0.77

For continuous variables (age): *P* value for Welch's ANOVA and *post hoc* Holm test
For categorical variables (sex): *P* value for Fisher's exact test
*$P<0.05$, **$P<0.01$

median, 11.5 years; Fig. 1). The 20% trimmed mean was 14.2 years (SE=2.2).

The lesions were classified as cavernous lymphangioma, cystic hygroma, cutaneous lymphangioma, intraabdominal lymphangioma, and other miscellaneous types. Cutaneous lymphangioma was further classified into superficial (lymphangioma circumscriptum) and deep. Baseline clinical and pathological characteristics are summarized in Tables 1 and 2, respectively. The anatomical location of lymphangiomas is shown in Table 3.

Cavernous lymphangioma

Fifty-eight cases were classified as cavernous lymphangioma. They were located in subcutaneous tissue and skeletal muscle. There was no difference in tissue localization among cavernous lymphangiomas of the different locations. Clinically, cavernous lymphangioma of the tongue and lip presented as macroglossia and macrocheilia. The age range of patients with cavernous lymphangioma at the time of diagnosis was 0 to 73 years (mean, 15.8 years; median, 9 years). The 20% trimmed mean age was 13.2 years (SE=2.4). Welch's *t* test revealed a significant difference between the age of male patients (mean, 8.9 years; median, 5 years) and that of female patients (mean, 22.8 years; median, 17 years; *P*=0.0018). The 20% trimmed mean ages of male and female patients were 6.3 years (SE=1.6) and 19.0 years (SE=4.4), respectively.

Gross findings showed that a sponge-like mass consisted of numerous microcysts. Histopathologically, irregular interconnecting lymphatic vessels with a thin and discontinuous smooth muscle layer were seen. Lymphatic spaces contained proteinaceous material, occasional lymphocytes, and few red blood cells. Supporting stroma was composed of collagen. In stroma-rich areas, microscopic lymphatic channels frequently lacked a muscle layer. In some cases, lymphoid aggregates were observed (Fig. 2a).

Five cases (9%) showed prominent cystic change and were classified as cystic variants of cavernous lymphangioma. Gross and histopathological findings showed large multiple cysts in the main mass. The sites involved by this variant were extremities or the lower abdominal region, distinguishable from cystic hygroma, which occurs in the cervicofacial and thoracic areas. Eleven cases (19%) had

Table 2 Comparison of pathological characteristics

Subtypes	Characteristics
Cavernous lymphangioma	Consists of irregular interconnecting lymphatic vessels with a thin and discontonuous smooth muscle layer. In stoma-rich areas, lymphatic channels frequently lack a muscle layer. Lymphoid aggregates may be accompanied.
Cystic variant	Prominent cystic change is seen in the main mass of cavernous lymphangioma.
Accompanying superficial lymphangioma	Occurs on the skin overlying the main mass of cavernous lymphangioma. Hispathological findings are similar to lymphangioma circumsriptum.
Cystic hygroma	Occurs exclusively in the cervicofacial, axillary and pectoral regions. It consists of one or more interconnecting large cysts with a thin wall. Lymphoid aggregates may be accompanied.
Cutaneous lymphangioma	
Lymphangioma circumscriptum	Consists of dilated capillary lymphatic vessels in the papillary dermis. Large vessels are not involved. The overlying epidermis is acanthotic and papillomatous.
Deep type	Locates in the dermis. Similar to but smaller than cavernous lymphangioma. No epidermal involvement.
Intraabdominal lymphangioma	Intraabdominal counterpart of cavernous lymphangioma. It consists of irregular interconnecting lymphatic vessels with a thin and discontinuous smooth muscle layer.

Table 3 Anatomical location of a series of lymphangiomas (114 cases)

Subtypes & anatomical location	Number (%)	Subtotal (%)	Total (%)
Cavernous lymphangioma			58 (51.8%) (11[a], 5[b])
Head and Neck		20 (17.9%) (4[a])	
Cheek	2 (1.8%)		
Lip	3 (2.7%)		
Tongue	4 (3.6%) (3[a])		
Oral cavity	1 (0.9%) (1[a])		
Submandibular region	4 (3.6%)		
Cervical region	6 (5.4%)		
Trunk		16 (14.3%) (2[a], 2[b])	
Pectoral region	4 (3.6%)		
Mediastinum	1 (0.9%)		
Axillary region	4 (3.6%) (2[a])		
Abdominal region	3 (2.7%) (1[b])		
Inguinal region	1 (0.9%) (1[b])		
Back	2 (1.8%)		
Cauda equina	1 (0.9%)		
Upper limb		9 (8.0%) (2[a], 1[b])	
Arm	3 (2.7%) (1[a])		
Forearm	3 (2.7%) (1[b])		
Hand/Thumb/Finger	3 (2.7%) (1[a])		
Lower limb		13 (11.6%) (3[a], 2[b])	
Buttock	1 (0.9%)		
Thigh	8 (7.1%) (2[a], 2[b])		
Popliteal region	1 (0.9%)		
Leg	3 (2.7%) (1[a])		
Cystic hygroma			28 (25.0%)
Submandibular/Cervical/ Axillary/Pectoral regions	28 (25.0%)		
Cutaneous lymphangioma			14 (10.7%) (2[c])
Cheek	1 (0.9%) (1[c])		
Nuchal region	1 (0.9%)		
Axillary region	2 (1.8%)		
Abdominal region	1 (0.9%)		
Lumbar region	1 (0.9%)		
Vulva	4 (3.6%)		
Forearm	1 (0.9%)		
Thigh	1 (0.9%)		
Foot	2 (1.8%) (1[c])		
Intraabdominal lymphangioma			10 (8.9%)
Stomach	1 (0.9%)		
Ileum	2 (1.8%)		
Colon	3 (2.7%)		
Retroperitoneum	4 (3.6%)		
Others			4 (3.6%)
Epicardium	1 (0.9%)		
Lung	1 (0.9%)		
Vertebra	2 (1.8%)		

[a] Number of cavernous lymphangioma with superficial lymphangioma
[b] Number of cystic variant of cavernous lymphangioma
[c] Number of deep type of cutaneous lymphangioma

superficial components resembling lymphangioma circumscriptum. They presented with papules on the skin overlying the main mass of cavernous lymphangioma in deep soft tissue. Histopathologically, these superficial lesions consisted of dilated capillary lymphatic vessels in the papillary dermis. The overlying epidermis was acanthotic and papillomatous.

Cystic hygroma

Twenty-eight cases were classified as cystic hygroma. These were exclusively located in the cervicofacial, axillary, and pectoral regions. In two cases, lesions extended into the mediastinum. The age range at the time of diagnosis was 0 to 44 years (mean, 8.5 years; median,

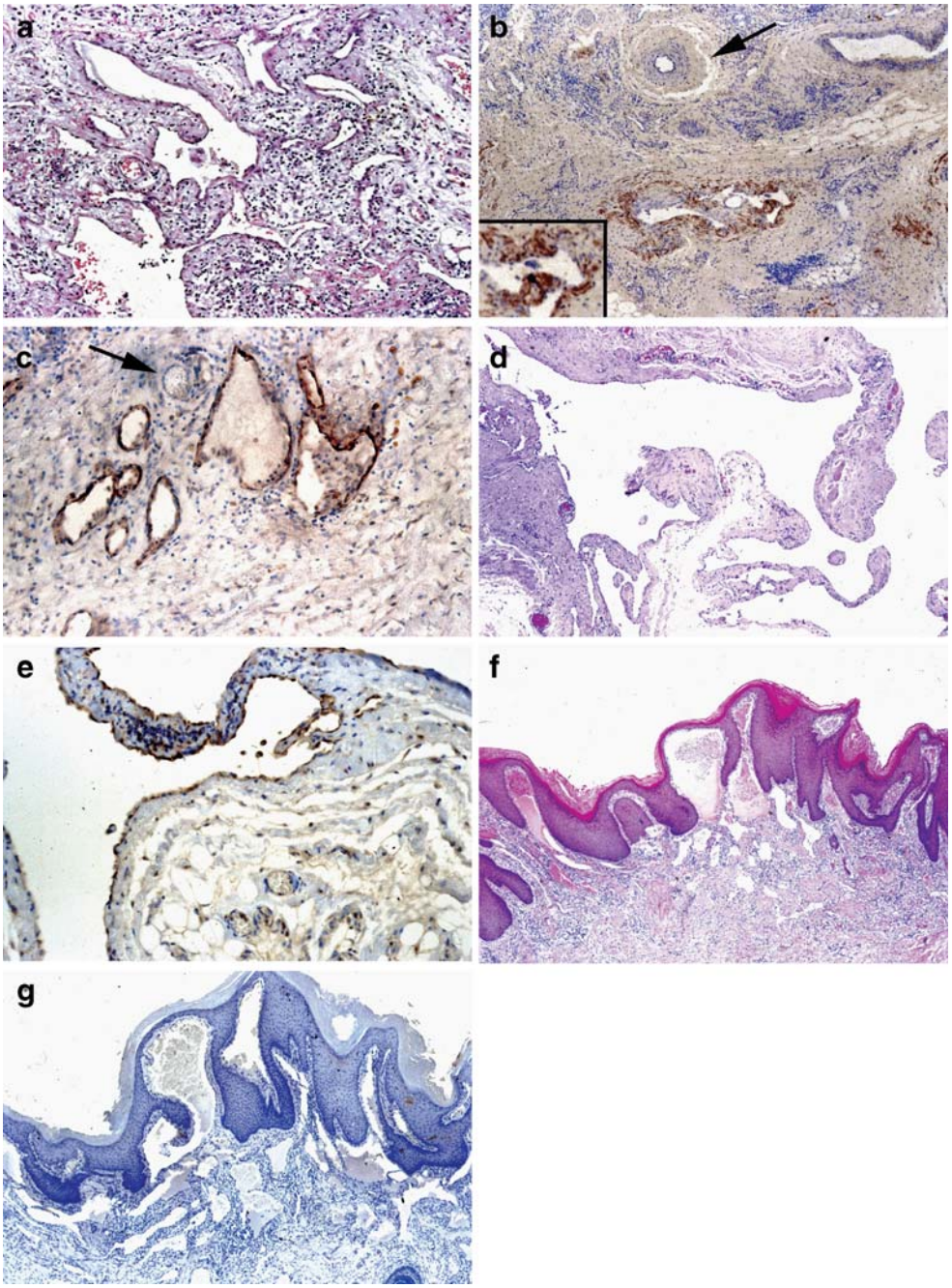

Fig. 2 Cavernous lymphangioma (**a**–**c**). **a** Irregular interconnecting lymphatic channels are located in the collagenous stroma. Lymphoid aggregates are also observed. **b** Strong reactivity for VEGF-C is seen in the lymphatic endothelium and in the smooth muscle cells of irregularly shaped lymphatic vessels. Immunoreactivity for VEGF-C is not detected in normal existing blood vessels (*arrow*). **c** Strong reactivity for VEGFR-3 is seen in the lymphatic endothelium. Immunoreactivity for VEGFR-3 is absent in normal existing blood vessels (*arrow*). Cystic hygroma (**d**–**e**). **d** Cystic lymphatic spaces with a thin and discontinuous smooth muscle layer. **e** Immunoreactivity for VEGFR-3 is detected in the endothelium lining the large cystic lymphatic spaces. Lymphangioma circumscriptum **f**–**g**. **f** The protuberant lesion consists of dilated capillary lymphatic vessels in the papillary dermis. **g** Immunoreactivity for VEGF-C is absent in the superficial lesion

3 years). The 20% trimmed mean age was 4.1 years (SE=1.5). Five cases occurred in adults. Clinical characteristics of adult cystic hygroma are shown in Table 4. There was one case of recurrence 38 years after operation at the age of 6 years. The other adult cases were seen as a new development. There was no significant difference between the age of male patients (mean, 11.8 years; median, 6.5 years) and that of female patients (mean, 5.3 years; median, 1 year; $P>0.05$). The 20% trimmed mean ages of male and female patients were 8.3 years (SE=3.6) and 1.3 years (SE=0.7), respectively.

Gross findings showed one or more interconnecting large cysts with a thin cyst wall. Histopathologically, large irregular lymphatic vessels were seen. The layer of smooth muscle was thin and discontinuous (Fig. 2d). Lymphoid aggregates were occasionally observed. Cavernous lymphangiomas occurring in the head and neck or in the trunk were distinguished from cystic hygroma based on the

Table 4 Clinical features of adult cystic hygroma

Case	Age (years)	Sex	Site of lesion	Previous history
1	18	F	Neck	
2	24	M	Neck	
3	37	M	Mediastinum	
4	43	F	Neck	
5	44	M	Chest wall	Surgery for cystic hygroma at the age of 6 years

predominant presence of microscopic cysts in cavernous lymphangiomas.

Cutaneous lymphangioma

Cutaneous lymphangiomas were classified as superficial and deep. The former represents lymphangioma circumscriptum. In the latter, only the deep dermis was involved without epidermal or superficial dermal changes.

Superficial type (lymphangioma circumscriptum) Twelve cases were classified as lymphangioma circumscriptum. The age range was from 9 to 79 years (mean, 37.1 years; median, 34 years). The 20% trimmed mean age was 34.5 years (SE=8.6). The age of patients with lymphangioma circumscriptum was significantly higher than that of patients with superficial lymphangioma associated with cavernous lymphangioma (mean, 18.5 years; median, 17 years; 20% trimmed mean, 18.1 years [SE=4.6]) (P= 0.022). The patients with lymphangioma circumscriptum presented with papules on the skin. Histopathologically, the lesions consisted of dilated capillary lymphatic vessels positioned within the papillary dermis. Large vessels were not involved. The overlying epidermis was acanthotic and papillomatous (Fig. 2f).

Deep type Two cases were classified as this subtype, one in the cheek, the other in the foot. These presented with small nodules in skin. The histopathological features were similar to cavernous lymphangioma. Irregular interconnecting lymphatic vessels with a thin and discontinuous muscular layer were seen, limited to the deep dermis. Deep type cutaneous lymphangiomas were distinguished from lymphangioma circumscriptum based on absence of superficial dermal lesions and lack of hyperplastic epidermal changes in the deep type.

Intraabdominal lymphangioma

Ten cases were classified as intraabdominal lymphangioma. The age range of patients with intraabdominal lymphangioma at the time of diagnosis was 0 to 62 years (mean, 38.8 years; median, 45 years). The 20% trimmed mean age was 42.5 years (SE=7.1). Gross and histopathological findings were similar to those of cavernous lymphangioma. The lesion consisted of irregular lymphatic vessels with a thin and discontinuous smooth muscle layer.

Others

There were two patients with lymphangioma of the spine and another with lung. The pathological features were similar to those of typical cavernous lymphangioma. A lymphangioma of the epicardium was also identified, composed of relatively uniform microscopic cysts.

Of three cases of intestinal lymphangiectasia, two were from the jejunum and one from the ileocecal valve. The age range of patients with intestinal lymphangiectasia was 55 to 62 years.

Welch's ANOVA revealed a highly significant difference in mean age among cavernous lymphangioma, cystic

Table 5 Results from the immunohistochemical analysis

	VEGF-C	(row %, reactivity: number of cases)		VEGFR-3	(row %, reactivity: number of cases)
Cavernous lymphangioma (excluding the cystic variant)	21/53	(40%, 1+:13, 2+:7, 3+:1)	**	38/53	(72%, 1+:21, 2+:9, 3+:8)
Cystic hygroma	10/28	(36%, 1+:9, 2+:1)	*	20/28	(71%, 1+:13, 2+:3, 3+:4)
Lymphangioma circumscriptum	0/12	(0%)	*	6/12	(50%; 1+:4, 2+:1, 3+:1)
Intra-abdominal lymphangioma	4/10	(40%, 1+:4)		6/10	(60%; 2+:2, 3+:4)
P value	*P*=0.035			*P*=0.45	

P value for Fisher's exact test
*$P<0.05$, **$P<0.01$

hygroma, lymphangioma circumscriptum, and intraabdominal lymphangioma (P=0.0002). The mean age of patients with lymphangioma circumscriptum was significantly higher than cavernous lymphangioma (P=0.034) or cystic hygroma (P=0.0065) by Holm–Bonferroni test. The mean age of patients with intraabdominal lymphangioma was significantly higher than cavernous lymphangioma (P=0.034) or cystic hygroma (P=0.0081) by Holm–Bonferroni test.

Immunohistochemical findings

Immunoreactivity for VEGF-C was detected in lymphatic endothelium and smooth muscle (Fig. 2b). VEGFR-3 was found exclusively in lymphatic endothelium (Fig. 2c). VEGF-C and VEGFR-3 were specific to lymphatic lineage and were not detected in blood vessels (Fig. 2b, c). Results from the immunohistochemical analysis are summarized in Table 5.

In cavernous lymphangioma including the cystic variant, VEGF-C and VEGFR-3 were detected in 21 of 58 cases (36%) and 41 of 58 cases (71%), respectively. Spearman's coefficient between VEGF-C and VEGFR-3 was 0.31 (P= 0.027), suggesting that they were mildly correlated at a significant level. There was no significant correlation between age and either VEGF-C or VEGFR-3. In cavernous lymphangioma excluding the cystic variant, VEGF-C and VEGFR-3 were detected in 21 of 53 cases (40%) and 38 of 53 cases (72%), respectively. In the cystic variant (five cases), VEGFR-3 was detected in three of five cases (60%), but VEGF-C was not detected. There was no significant difference in the reactivity of VEGF-C or VEGFR-3 between male and female patients. VEGF-C was absent from the superficial lymphangioma when associated with cavernous lymphangioma (11 cases). VEGFR-3 was detected in the lymphatic endothelium of the lesion in seven of 11 cases (64%).

In cystic hygroma, VEGF-C and VEGFR were detected in ten of 28 cases (36%) and 20 of 28 cases (71%), respectively. In positive cases, VEGF-C expression was patchy and focal in the lymphatic vessel wall and the positivity score was limited to 1+ except for one case. VEGFR-3 was detected in the lymphatic endothelium of the large cystic lymphatic vessels (Fig. 2e). The degree of expression of VEGFR-3 varied among the cases. Spearman correlation coefficients between age and either VEGF-C or VEGFR-3 were not significant.

In lymphangioma circumscriptum, VEGFR-3 was detected in the lesional endothelium in six of 12 (50%), but VEGF-C was not detected in all cases (Fig. 2g). In the deep type, VEGF-C was detected in one of two cases. VEGFR-3 was detected in both cases.

In intraabdominal lymphangioma, VEGF-C was detected in lesional cells in four of ten (40%). In positive cases, VEGF-C expression was patchy and focal and of limited extent (reactivity, 1+). VEGFR-3 was detected in the lesional endothelium in six of ten (60%). In intestinal lymphangiectasia, VEGFR-3 was detected in the lesional endothelium in two of three (67%), but VEGF-C was absent in all cases.

In the lung lymphangioma, immunoreactivity was detected for both VEGF-C (reactivity, 3+) and VEGFR-3 (reactivity, 3+). In the epicardial lymphangioma, immunoreactivity was also detected for both VEGF-C (reactivity, 2+) and VEGFR-3 (reactivity, 2+). In both vertebral lymphangiomas, neither VEGF-C nor VEGFR-3 was detected.

Fisher exact test revealed a highly significant difference in the positivity of VEGF-C among the four groups (P= 0.0035) as well as between lymphangioma circumscriptum and cavernous lymphangioma excluding the cystic variant (P=0.0063), cystic hygroma (P=0.019), or intraabdominal lymphangioma (P=0.029). There was no significant difference in the positivity of VEGFR-3 among these groups.

Discussion

Lymphangiomas are generally regarded as vascular malformations [4, 7] occurring during embryonic development when VEGF-C and VEGFR-3 are central regulators. Peak VEGF-C expression is seen during the earlier stages of lymphangiogenesis with much less expression during lymphatic capillary organization and functional integration. After embryonic development, VEGF-C expression decreases in most tissues, remaining high in the lymph nodes [8, 19]. VEGF-C promotes survival of the VEGFR-3-expressing lymphatic endothelial cells via the MAPK signaling pathway [20]. However, the role of VEGFR-3 signaling is not very important for the survival of mature lymphatic vessels [21]. Although VEGFR-3 is mostly regarded as a lymphatic marker, it is not exclusive to the lymphatic lineage. VEGFR-3 is exceptionally expressed in blood vascular endothelium during embryogenesis and is essential in the development of the major cardiovascular system before the emergence of the lymphatic vessels [22]. VEGFR-3 may also be present, though to a less extent, in some blood vascular tumors including angiosarcoma [17, 23, 24]. This receptor has been detected in virtually all Kaposi's sarcomas [23–25] which are consequently thought to originate from lymphatic endothelium.

In this study, we found that the expression of the factor and its receptor was different among the various types. VEGF-C was detected in lymphatic endothelium and smooth muscle in 36% cavernous lymphangioma. Positivity was moderately correlated to VEGFR-3 expression, suggesting they both contribute to the development of this lesion, possibly by autocrine and paracrine regulation [13].

VEGFR-3 was detected in 72% of cavernous lymphangiomas. The receptor was expressed in both small and dilated lymphatics, in contrast to D2-40, another lymphatic endothelial cell-specific marker recognizing M2A antigen which has a tendency for better positivity in small lymphatic channels [26]. We hypothesize that VEGF-C and VEGFR-3 might be preferentially expressed in early life and the expression of VEGF-C and VEGFR-3 would tend to decrease in older patients. However, in our study, there was no significant correlation between the immunoreactivity and patient's age. Strong expression of VEGF-C and VEGFR-3 was detected even in older adult patients. Although it is widely regarded as a hamartomatous lesion rather than a true neoplasia, VEGF-C and VEGFR-3 seem to contribute to proliferation in certain cases of cavernous lymphangioma, which may account for some cavernous lymphangiomas being more active and possibly neoplastic, especially in older adult patients.

Cystic hygroma, also called macrocystic lymphatic malformation of the head and neck, is believed to be a consequence of disruption of normal lymphatic development at the lymphovenous connection as a result of defective genes. It is considered that a small portion of the jugular lymphatic sac located laterally in the neck is sequestrated during embryologic life and later becomes cystically dilated. This lesion has been associated with aneuploid chromosomal abnormalities, particularly Turner's syndrome, with poor survival [27–29]. Cystic hygroma is usually present at birth and can be diagnosed by prenatal ultrasound. Spontaneous regression may be observed *in utero*, but this does not preclude abnormal karyotype or fetal anomalies [28]. Meanwhile, cystic hygroma is very rarely seen in adults. It is considered highly unusual for cystic hygroma to present after the fourth decade of life, although there are a few reports in the literature [30–34]. Recurrence after resection is uncommon, but it may occur within a few years. There are only a few case reports of recurrence after more than 20 years [35, 36]. In our study, VEGF-C expression by lesional cells of cystic hygroma was more focal and limited in its distribution. Cystic hygroma may be responsive to VEGF-C, but increase in size may only follow accumulation of fluid after lymphatic sequestration.

It has been proposed that lymphangioma circumscriptum represents dilatation of peripheral lymphatics secondary to absent, inadequate, or obstructed efferents [37]. In our study, VEGF-C was absent, and this growth factor does not seem to be associated with the development of lymphangioma circumscriptum. It may be the lymphatic counterpart of angiokeratoma, which is of a telangiectatic nature shared by different disease entities. The histological features resemble each other and share ectatic vessels in the papillary dermis with overlying hyperplastic epidermis. The nature of this superficial lymphangioma is more likely considered to be lymphangiectatic.

Lymphangiomas in deep soft tissue may accompany superficial lesions in overlying skin [38] or mucosa. In our series, the superficial lesion was also recognized in 11 cases (19%) of cavernous lymphangioma. VEGF-C was absent in the superficial lesion even in the cases in which the main deep component expresses VEGF-C. The superficial lesion may also only follow chronic lymphatic stasis.

Intraabdominal lymphangiomas have been considered the counterpart of cavernous lymphangioma in the trunk or extremities [4]. However, patients with intraabdominal lymphangioma were significantly older than those with cavernous lymphangioma or cystic hygroma. Intraabdominal lymphangioma is apt to be diagnosed later in life, partly because it is clinically less significant. VEGF-C was detected in 40% cases, and in the positive cases, its expression was focal and limited in its distribution. Previous cell proliferation analysis revealed that intraabdominal lymphangiomas are quiescent, manifesting low activity [39]. Enlargement of intraabdominal lymphangiomas may be due to engorgement by chyle and localized secondary reaction, and the contribution of the growth factor seems limited.

In pulmonary lymphangioma, despite a report that the proliferation activity of this type was low [40], our case showed strong VEGF-C reactivity, suggesting high proliferative activity. In bone lymphangioma, expression of VEGF-C and VEGFR-3 was negative. However, immunoreactivity may have been abolished by decalcification.

In summary, we have demonstrated that the expression of VEGF-C was different in the various types of lymphangioma. In some typical cases of cavernous lymphangioma, VEGF-C was strongly expressed, suggesting that these cases possessed proliferative activity. In cystic hygroma and intraabdominal lymphangioma, the expression of VEGF-C was focal and limited in its distribution. VEGF-C was absent from lymphangioma circumscriptum and superficial lymphangiomas associated with cavernous lymphangiomas. The nature of superficial lymphangiomas is more likely considered to be lymphangiectatic.

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Conflict of interest We declare that we have no conflict of interest.

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