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Original Article

Expression of cancer stem cell markers and their correlation with pathogenesis in vascular tumors

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Abstract: Objective: Vascular tumor, which belongs to a kind of complicated lesion in soft tissue tumor, is derived from mesenchymal tissue. Although many studies have been focused on the pathogenesis of vascular tumors in human, the specific mechanism of the vascular tumors was currently unclear. Previous studies have reported an association of cancer stem cells with the development of tumor in many solid tumors. Thus the purpose of this study was to explore whether different expression level of cancer stem cell markers including CD29, CD44, CD133, nestin and ALDH1 in vascular tumor may help to elucidate the possible pathogenesis of vascular tumor. In present study, tissues of 9 cases of hemangioma, 22 cases of hemangiosarcoma, 3 cases of Kaposi's sarcoma, and 5 cases of hemangioendothelioma were immunostained for CD29, CD44, CD133, nestin and ALDH1. Of the 39 vascular tumor cases included in the current study, CD29, CD133 and nestin were positive in most vascular tumor cases. Although CD44 and ALDH1 were observed in vascular tumor cases, the percentage of cells staining for the two markers was less than 2% in all cases of vascular tumor. Capillary hemangiomas exhibited significantly higher expression rate of CD29 and nestin compared with malignant vascular tumors and hemangioendotheliomas ($P<0.05$, Fisher's exact test), while CD44, CD133 and ALDH1 exhibited no statistically significant difference between these two groups. Pearson correlation analysis exhibited that CD29 expression and nestin expression in vascular tumor were no statistically significant relationship ($C=0.288$, $P=0.063>0.05$). Our findings confirmed that the five cancer stem cells markers, including CD29, CD44, CD133, nestin and ALDH1, exhibited different expression levels in vascular tumors and demonstrated that immonhistochemical analysis for cancer stem cells markers may provide useful information for studying the pathogenesis of vascular tumors.

Keywords: Vascular tumor, immunohistochemistry, cancer stem cells, pathogenesis

Introduction

Vascular tumor is derived from mesenchymal tissue tumors, which is classified as soft tissue tumors [1, 2]. According to the 4th WHO classification, it can be divided into benign tumors of blood vessels, malignant vascular tumors, and hemangioendothelimas that are vascular tumor of intermediate malignancy. Hemangiomas are most common benign vascular tumor of childhood with a unique characteristic process of its rapid growth and slowly spontaneous involution [3-5]. The occurrences of malignant and potentially malignant sarcomas that originated from blood vessels are rarely seen and include hemangiosarcomas [6, 7], Kaposi's sarcomas [8], hemangioendothelimas [9, 10], usually with no known cause. The only known reasons of hemangiosarcomas were a few rare specific genetic alterations such as anti-oncogene (p53) mutation, previous irradiation, and exposure to vinyl chloride or thorotrast [11, 12]. Kaposi's sarcomas often occurred in patients with infection and patients who are immunosuppressed [13, 14]. Although there were several previous studies have paid more attentions on the pathogenesis and origin of vascular tumors, it still remains controversial and was not well understood in humans.

Increasing evidence showed that tumors may derive from a small subpopulation of cancer cells possessing stem-like properties, which were defined as cancer stem cells or tumor-ini-

Table 1. Primary antibodies used in the immunohistochemistry

Antigen	Location	Antibody species	Manufacturer	Clone Number	Antigen-retrieval solution	Dilution
CD133	C	Rabbit polyclonal	ARP, Waltham, America	05-PA1021	PCA-CB	1:200
CD29	C	Rabbit monoclonal	Abcam, Cambridge, UK	EP1041Y	PCA-CB	1:600
CD44	M/C	Mouse monoclonal	DAKO, Glostrup, Denmark	DF1485	PCA-CB	1:300
Nestin	C	Rabbit monoclonal	Abcam, Cambridge, UK	SP103	PCA-CB	1:800
ALDH1	C	Rabbit monoclonal	Abcam, Cambridge, UK	EP1933Y	PCA-CB	1:300

C: cytoplasm, M: membrane, PCA-CB: pressure cooker heating in citrate buffer (0.01 M, pH 6.0).

Table 2. Summary of patients' clinical information in benign vascular tumors

Patient ID	Tumor	Age	Sex	Location
1	Capillary hemangioma	7 m	F	Left shoulder
2	Capillary hemangioma	16 m	F	Lower abdomen and perineum
3	Capillary hemangioma	3 y	M	Back buttocks
4	Capillary hemangioma	8 m	F	Neck
5	Capillary hemangioma	2 y	F	Left upper eyelid
6	Capillary hemangioma	8 m	M	Scalp
7	Capillary hemangioma	7 m	F	Right thigh, right chest subcutaneous
8	Capillary hemangioma	7 m	F	Scalp
9	Capillary hemangioma	25 y	M	Nose

F, female; M, male; m, month; y, year.

tiating cells [15, 16]. The cancer stem cell hypothesis proposes that cancer stem cells may be involved in tumor progression and several tumor characteristics, such as recurrence and metastasis [17, 18]. These cells with the potential to initiate and maintain tumor development were first identified in leukemia [19] and subsequently in breast cancer [20], colon cancer [21], sarcoma [22, 23] et al. Specific surface markers can help to identify and isolate the cancer stem cells [24, 25]. Recent studies have reported that only a small population of tumor cells can selectively express certain surface markers such as CD29 [26], CD44 [27, 28] and CD133 [29]. Additionally, it was reported that vascular tumors also expressed cancer stem cell markers, indicating an association of cancer stem cell with the initiation and pathogenesis of vascular tumors [3, 6, 11]. Thus, the importance of identifying cancer stem cell for understanding of the pathogenesis and origin of vascular tumors is becoming more evident.

In the present study, we investigated the different expression of cancer stem cell markers including CD29, CD44, nestin, CD133 and ALDH1 in resected specimens obtained from 39 cases of vascular tumor tissues by immunohischemistry. Our results allowed us to define the phenotype of cancer stem cells in different vascular tumors, which may contribute to our understanding of the pathogenesis and origin of vascular tumor.

Material and methods

Patients and tumor specimens

The study included 9 cases capillary hemangioma, 25 cases of malignant vascular tumor (including 3 cases of Kaposi's sarcoma and 22 cases of hemangiosarcoma). In addition, 5 cases of hemangioendothelioma were identified. A total of 39 cases were obtained from the Department of Pathology, the First Affiliated Hospital, Shihezi University School of Medicine. Tumor tissues were fixed in buffered formalin and subjected to routine processing and paraffin embedding. Clinical data including age, sex, and location of tumor were available in all cases (**Tables 2**, **3**). This study was conducted with the approval of the institutional ethics committee at the First Affiliated Hospital of Shihezi University of Medicine.

Immunohistochemical staining

The most representative paraffin blocks were identified by examination of hematoxylin and

Table 3. Summary of patients' clinical information in malignant vascular tumors

Patient ID	Tumors	Age/year	Sex	Location
1	Hemangioendothelioma	4 m	M	Left of face
2	Hemangioendothelioma	68	F	Right nasal septum
3	Kaposiform hemangioendothelioma	29	F	Head
4	Hemangioendothelioma	30	M	Right femur
5	Hemangioendothelioma	43	M	Penis
6	Epithelioid hemangiosarcoma	28	F	Scalp
7	Epithelioid hemangiosarcoma	62	M	Gingiva
8	Epithelioid hemangiosarcoma	69	M	Right prefrontal
9	Epithelioid hemangiosarcoma	51	F	Right wing fossa
10	Epithelioid hemangiosarcoma	76	M	Scalp
11	Epithelioid hemangiosarcoma	76	M	Head
12	Epithelioid hemangiosarcoma	45	M	Head
13	Hemangiosarcoma	68	F	Breast
14	Hemangiosarcoma	83	F	Scalp
15	Hemangiosarcoma	73	F	Head
16	Hemangiosarcoma	43	F	Right neck
17	Hemangiosarcoma	48	M	Gingiva
18	Hemangiosarcoma	29	M	Spleen
19	Hemangiosarcoma	59	F	Popliteal fossa
20	Hemangiosarcoma	59	M	Lower limb skin
21	Hemangiosarcoma	65	M	Armpit
22	Hemangiosarcoma	59	F	Breast
23	Hemangiosarcoma	64	M	Armpit
24	Hemangiosarcoma	63	M	Upper limb
25	Hemangiosarcoma	62	M	Upper limb
26	Hemangiosarcoma	36	F	Breast
27	Hemangiosarcoma	56	M	Left leg
28	Kaposi's sarcoma	25	M	Right scapula
29	Kaposi's sarcoma	84	M	Right hand, right lower limb skin
30	Kaposi's sarcoma	ND	F	Left index finger

F, female; M, male; m, month; ND, no data.

Table 4. Immunohistochemical staining results for different markers in benign vascular tumor

Patient ID	Age	Sex	Cancer stem cell markers				
			CD29	CD44	CD133	Nestin	ALDH1
1	7 m	F	+	-	+	++	-
2	16 m	F	++	+	+	+	-
3	3 y	M	+	+	-	+	-
4	8 m	F	+	-	++	++	-
5	2 y	F	+	-	++	++	-
6	8 m	M	++	-	++	++	-
7	7 m	F	+	-	+	+	-
8	7 m	F	++	+	+	+	-
9	25 y	M	++	-	+	++	-

F, female; M, male; m, month; y, year; -, negative; +, weak; ++/+++, strong.

eosin-stained slides. 4 μm serial tissue sections from formalin-fixed and paraffin-embedded tissue blocks of all tumors were obtained. Envisions two-step immunohistochemical kit (Dako system, Glostrup, Denmark) were available for detecting specific target proteins. Paraffin-embedded sections were heated at 58~60°C for 30 min. Then baked slides were deparaffinized in xylene and rehydrated in a graded series of alcohols. Antigen retrieval was performed by heat-induced in citrate puffer, PH 6.0. The condition of retrieval depended on each of the five different antibodies employed. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide for 10 min. Sections were

Table 5. Immunohistochemical staining results for markers in malignant lesions in vascular tumor

Patient ID	Age/ year	Sex	Cancer stem cell markers				
			CD29	CD44	CD133	Nestin	ALDH1
1#	4 m	M	-	-	+	+	-
2#	68	F	-	-	++	-	-
3#	29	F	-	-	+	-	-
4#	30	M	-	-	+	-	-
5#	43	M	-	-	+	-	-
6	28	F	+++	-	++	-	+++
7	62	M	+	-	-	++	-
8	69	M	+	-	-	-	-
9	51	F	++	-	-	+	-
10	76	M	-	-	-	-	+
11	76	M	+	-	-	++	-
12	45	M	+	-	-	-	-
13	68	F	++	-	-	+	-
14	83	F	+	+	-	-	-
15	73	F	++	+	-	-	-
16	43	F	-	+	+	-	-
17	48	M	-	-	+	++	-
18	29	M	++	-	-	++	+
19	59	F	+	-	-	+++	-
20	59	M	+++	-	++	-	++
21	65	M	+	++	+	+	-
22	59	F	-	-	+	-	-
23	64	M	++	-	++	-	-
24	63	M	-	-	+	-	-
25	62	M	-	-	+	+	+
26	36	F	++	-	+	-	-
27	56	M	+++	-	-	-	-
28*	25	M	+	-	+	+++	-
29*	84	M	++	-	-	-	-
30*	ND	F	+	-	+	++	-

F, female; M, male; m, month; ND, no data; -, negative; +, weak; ++/+++, strong. #Patient was diagnosed with intermediate vascular tumor; *Patient was diagnosed with Kaposi's sarcoma.

then incubated with primary antibodies (**Table 1**) for at least 8 hours at 4°C and PBS was instead of the primary antibodies for negative controls. PBS was used to wash the primary antibodies followed by the appropriate secondary antibodies (**Table 1**) for 30 min at 37°C, and reaction was performed using 3.3'-diaminobenzidine peroxidase substrate kit (Dako System, Glostrup, Denmark). Finally, sections were counter-stained with hematoxylin, dehydrated and mounted in a neutral mounting medium. Immunohischemical procedures including antibodies and primary antibodies used in our study are summarized in **Table 1**.

Evaluation of immunohistochemistry

The evaluation of immunostaining was performed independently by two pathologists, both of whom were blinded to the clinical and pathological data. For CD29, CD133, nestin and ALDH1, only cytoplasmic staining was considered positive, whereas for CD44, membranous immunoreactivity was evaluated positive. Immunohistochemical result of full sections was scored by multiplying the staining intensity and percent positive cells from the same area. Staining intensity was classified into four groups: 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The percentage of positive cells that revealed tumor cells staining was evaluated by four grades: 0, positive staining in 5% or less of the tumor cells; 1, positive staining in 6% to 25% of the tumor cells; 2, positive staining in 26% to 50% of the tumor cells; 3, positive staining in 51% to 75% of the tumor cells; and 4, positive staining in 76% to 100% of the tumor cells. Staining intensity was graded as negative (-), weak (+) and strong (++/+++).

Statistical analysis

Statistical analysis was performed using SPSS version 17.0 statistical sofware. Qualitative variables were analyzed by Chi-square test or Fisher's exact test. Pearson correlation analysis was used to determine the relationship between CD29 and nestin expression over all cases. *P*-values of < 0.05 with two-tailed *P* values were considered to be statistically significant difference.

Results

Clinicopathological characteristics of vascular tumors and markers expression

Of the 39 cases included in this study, 9 cases were regarded as capillary hemangioma and were located in the skin (head and neck) and subcutaneous tissue (left shoulder, lower abdomen and perineum, back buttocks, right thigh, right chest subcutaneous) (**Table 2**), which

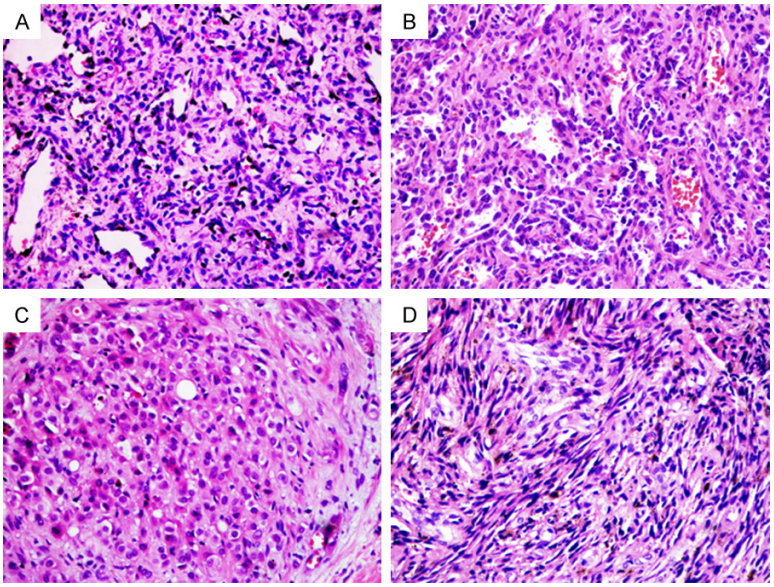

Figure 1. Vascular tumors stained by hematoxylin and eosin (H&E). Capillary hemangioma (A) showing mixture of mature and immature capillary vessels lined by flattened endothelium cells. Hemangiosarcoma (B) composed of irregular vascular channels lined by plump epithelioid endothelial cells. Hemangioedothelioma (C) composed of a small amount of round epithelioid cells with a hyaline cytoplasm. Kaposi's sarcoma (D) composed of mixed arrangement of spindle cells with hyperchromatic nuclei. Magnification, ×200.

including 6 women and 3 men with a median age of 8 months (range from 0.6 to 25 years). Immunohistochemical staining results for 5 markers on 9 patients with capillary hemangioma were presented in **Table 4**, respectively. 25 cases were tested with malignant vascular tumor and the median was 60.5 years (range from 25 to 84 years) including 10 women and 15 men, while these malignant tumors occurred in most parts of the body (**Table 3**). Immunohistochemical staining results for 5 markers on 25 patients with malignant vascular tumor were showed in **Table 5**, respectively. Additionally, 5 cases were diagnosed with intermediate malignancy of vascular tumors which including hemangioendothelioma (n=4) locating in the face, nasal septum, femur and penis, and Kaposiform hemangioendothelioma (n=1) that occurred in the head (**Table 3**) and the immunohistochemical staining results for 5 markers were presented in **Table 5**, respectively. Distribution of case numbers, age, sex, and location of tumors were listed in **Tables 2**, **3**. Representative hematoxylin and eosin (H&E)-stained histology slides from capillary hemangioma, hemangiosarcoma, hemangioendothelioma and Kaposi's sarcoma are shown in **Figure 1A-D**, respectively.

Expression of cancer stem cell markers CD29, CD44 and nestin in vascular tumors

28 of 39 vascular tumor cases were positive for CD29 (**Table 6**). CD29 was observed in the cytoplasm of vascular tumor tissue. CD29 positive tumor cells were presented in all capillary hemangioma cases (**Figure 2A** and **2B**). In contrast, CD29 was completely negative in all 5 hemangioendothelioma cases. 16 (72.7%) of 22 hemangiosarcoma cases (**Figure 3A** and **3B**) and 3 Kaposi's sarcoma case (**Figure 4A** and **4B**) were positive expression for CD29. Capillary hemangiomas cases showed significantly higher level of CD29 positive expression compared with malignant vascular tumors and hemangioendotheliomas (**Table 7**, P= 0.04, Fisher's exact test).

Of 39 vascular tumor samples which included in the present study, only 7 of cases were positive for CD44. Staining for CD44 showed a mixed membranous and cytoplasm pattern of staining in vascular tumors. 3 of 9 Capillary hemangioma cases were positive for CD44 (**Figure 2C** and **2D**). Only 4 of 25 CD44 staining was detected in 4 of 25 malignant vascular tumor including 22 hemangiosarcoma cases (**Figure 3C** and **3D**) and 3 Kaposi's sarcoma cases (**Figure 4C** and **4D**). CD44 was negative for all 5 hemangoendothelioma cases. Capillary hemangiomas showed no statistically significant difference compared with malignant vascular tumors and hemangioendotheliomas (**Table 7**, P=0.319, Fisher's exact test).

20 of 39 vascular tumor cases were positive for nestin (**Table 6**). Cytoplasmic staining for nestin was detected in vascular tumors, while 10 of 25 malignant vascular tumors including 22 hemangiosarcoma cases (**Figure 3E** and **3F**) and 3 Kaposi's sarcoma cases (**Figure 4E** and **4F**). Nestin-positive tumor cells were presented in all 9 Capillary hemangioma cases (**Figure 2E** and **2F**). Only 1 of 5 hemangioendothelioma cases were positive expression with weak staining. Capillary hemangiomas exhibited significantly higher expression rate of nestin com-

Table 6. CD29, CD44, CD133, Nestin, ALDH1 cancer stem cell markers expression in vascular tumors and staining intensity, respectively

	CD29-positive (%)				CD44-positive (%)				CD133-positive (%)				Nestin-positive (%)				ALDH1-positive (%)			
	1+	2+/3+	Total	Negative	1+	2+/3+	Total	Negative	1+	2+/3+	Total	Negative	1+	2+/3+	Total	Negative	1+	2+/3+	Total	Negative
HE	0	0	0 (5)	5 (5)	0	0	0 (5)	5 (5)	3	2	5 (5)	0 (5)	1	0	1 (5)	4 (5)	0	0	0 (5)	5 (5)
HAS	7	9	16 (22)	6 (22)	3	1	4 (22)	18 (22)	7	3	10 (22)	12 (22)	3	5	8 (22)	14 (22)	4	2	6 (22)	16 (22)
KAS	2	1	3 (3)	0 (3)	0	0	0 (3)	3 (3)	2	0	2 (3)	1 (3)	0	2	2 (3)	1 (3)	0	0	0 (3)	3 (3)
CH	5	4	9 (9)	0 (9)	3	0	3 (9)	6 (9)	5	3	8 (9)	1 (9)	4	5	9 (9)	0 (9)	0	0	0 (9)	9 (9)
Total	14	14	28 (39)	11 (39)	6	1	7 (39)	32 (39)	17	8	25 (39)	14 (39)	8	12	20 (39)	19 (39)	4	2	6 (39)	33 (39)

HE, hemangioedothelioma; HAS, hemangiosarcoma; KAS, Kaposi's sarcoma; CH, capillary hemangioma; ALDH1, aldehyde dehydrogenase 1; -, negative; 1+weak; 2+/3+ strong.

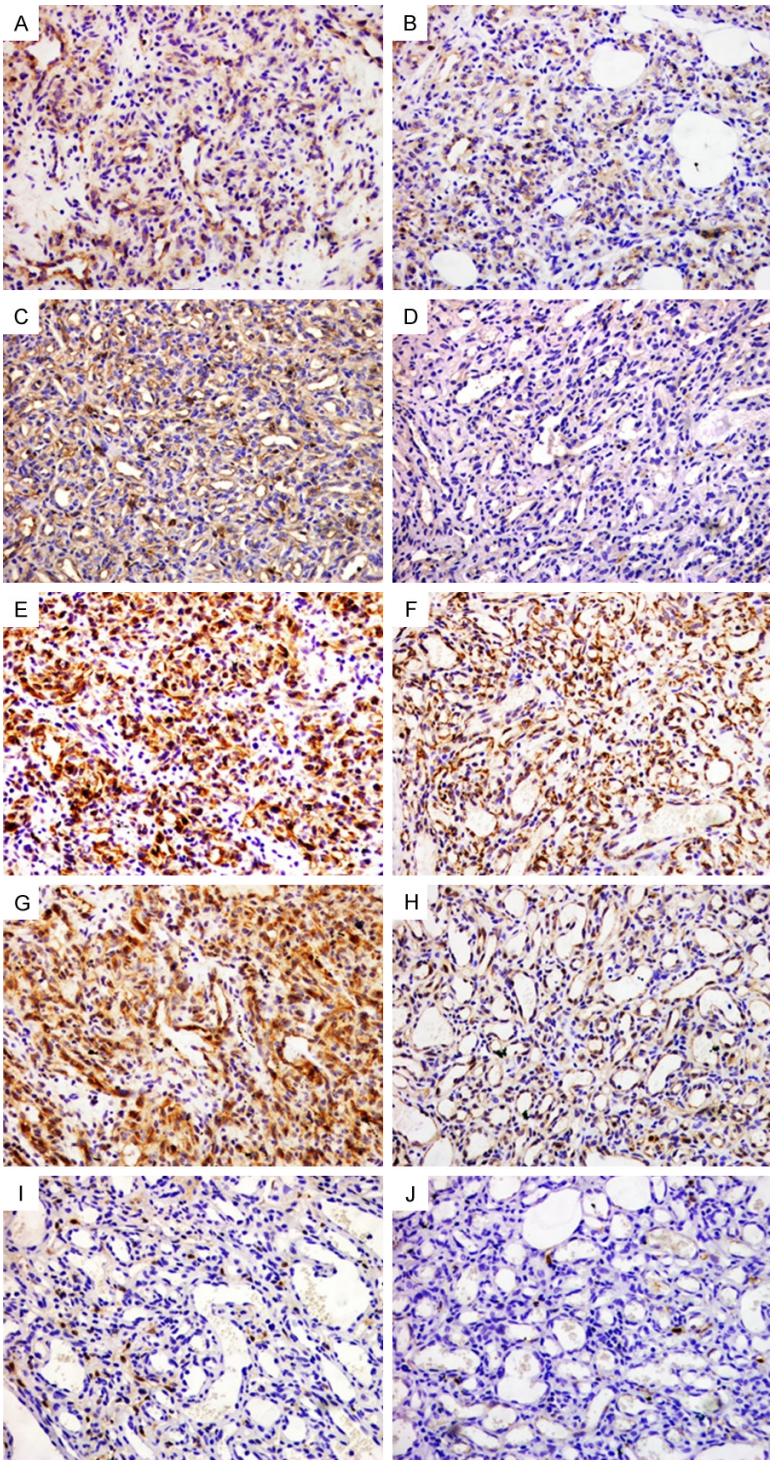

Figure 2. Representative immunohistochemical staining of CD29, CD44, nestin, CD133 and ALDH1 expression in capillary hemangioma tissues. Cytoplasmic positivity staining for CD29 (A and B) and nestin (E and F) was presented in all cases. CD44 was mainly immunostained in the cell membrane or cytoplasm near the cell membrane, positivity (C) and corresponding negativity (D) staining for CD44. Cytoplasmic positivity (G) and negativity (H) staining for CD133, all cases were negative stained for ALDH1 (I and J). Magnification, ×200.

pared with malignant vascular tumors and hemangioendotheliomas (**Table 7**, *P*= 0.001, Fisher's exact test).

Expression of cancer stem cell marker CD133 in vascular tumors

25 of 39 vascular tumor cases were stained with CD133 (**Table 6**). CD133 was detected in the cytoplasm of vascular tumor tissue. 8 of 9 capillary hemangioma cases were positive for CD133 (**Figure 2G** and **2H**). The percentage of tumor cells staining positive for CD133 in 12 (48%) of 25 malignant vascular tumor cases including 22 hemangiosarcoma cases (**Figure 3G** and **3H**) and 3 Kaposi's sarcoma cases (**Figure 4G** and **4H**). CD133 positive tumor cells were observed in five hemangioendothelioma cases. Capillary hemangiomas showed no statistically significant difference in CD133 expression compared with malignant vascular tumors and hemangioendotheliomas (**Table 7**, *P*>0.05, Fisher's exact test).

Expression of cancer stem cell marker ALDH1 in vascular tumors

Only 6 of 39 vascular tumor cases positive for ALDH1 staining with weak to strong intensity (**Table 6**), these cases were all hemangiosarcomas with cytoplasm stained (**Figure 3I** and **3J**). 9 Capillary hemangioma cases (**Figures 2I** and **3J**), 3 Kaposi's sarcoma cases (**Figure 4I** and **4J**), and 5 hemangioendothelioma cases were negative for ALDH1. Additionally, capillary hemangiomas showed no sta-

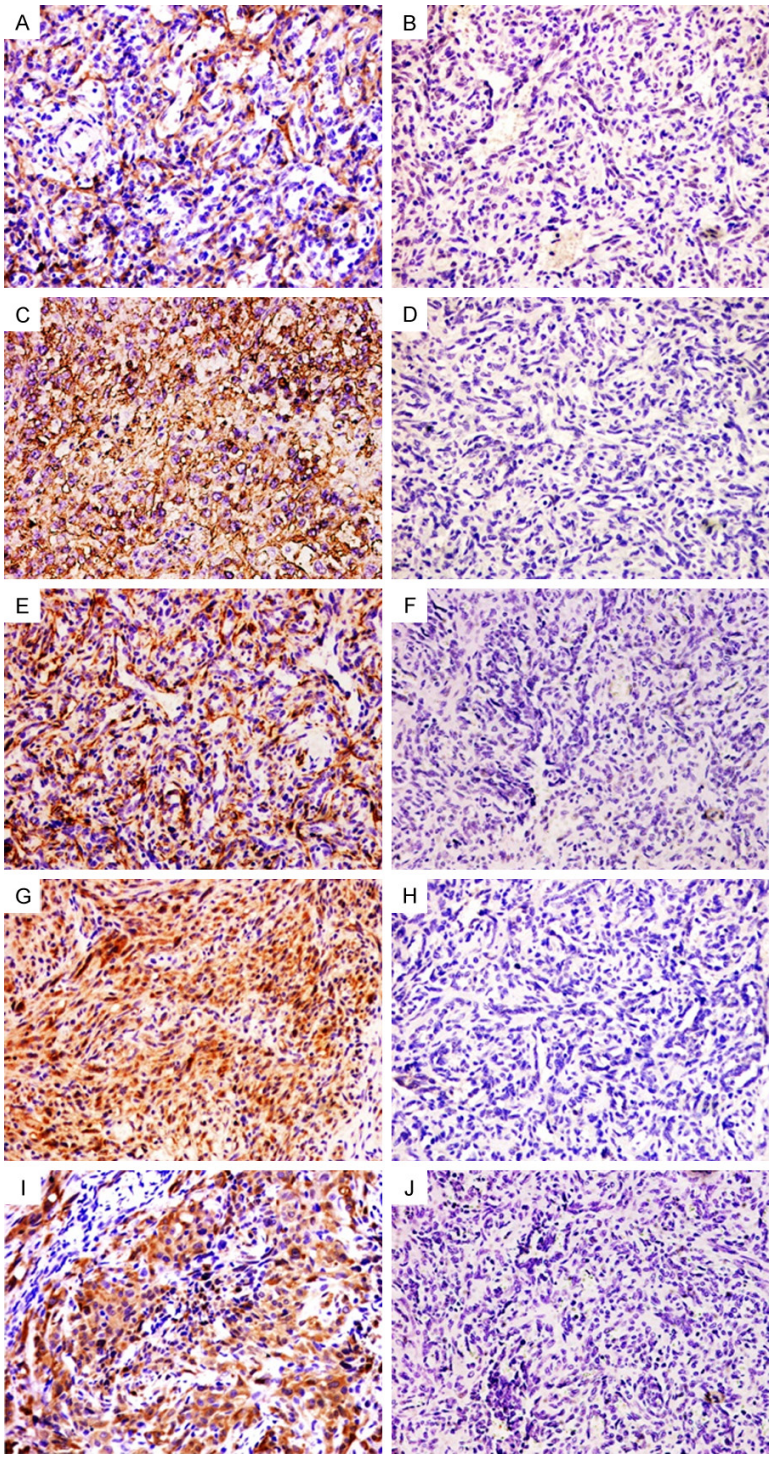

Figure 3. Representative immunohistochemical staining of CD29, CD44, nestin, CD133 and ALDH1 expression in hemangiosarcoma tissues. Hemangiosarcoma samples with cytoplasmic positivity and corresponding negativity for CD29 (A and B), nestin (E and F), CD133 (G and H), ALDH1 (I and J) and membranous positivity and corresponding negativity for CD44 (C and D). Magnification, ×200.

tistically significant difference compared with malignant vascular tumors and hemangioendothelioma (**Table 7**, *P*>0.05, Fisher's exact test).

Correlation between CD29 and nestin expression in vascular tumors

No statistically significant difference was detected between capillary hemangiomas and other types of vascular tumors including malignant vascular tumors and hemangioendotheliomas in CD133, CD44, and ALDH1 expression (*P*>0.05), while a statistically significant difference was found in CD29 and nestin expression (P<0.05). The positive rate for CD29 and nestin in 39 vascular tumor cases were 71.8% and 51.3%, respectively. Of the total 39 vascular tumor cases, CD29 positive and nestin positive expression was detected in 17 cases, while negative expression was detected in 8 cases. Additionally, 3 cases were positive for nestin, but negative for CD29. In contrast, 11 cases were observed CD29-positive and nestin-negative. Thus a correlation between CD29 and nestin expression in vascular tumors was analyzed by Pearson correlation analysis in this study. However, Pearson correlation analysis exhibited that CD29 and nestin expression in vascular tumors were no statistically significant relationship (**Table 8**, C=0.288, *P*>0.05).

Discussion

Vascular tumor, which is defined historically as a soft tumor of mesenchymal origin [2], is classified based on their histologic appearance and biological behavior. Several histologic subtypes of vascular tumor are known [1], including

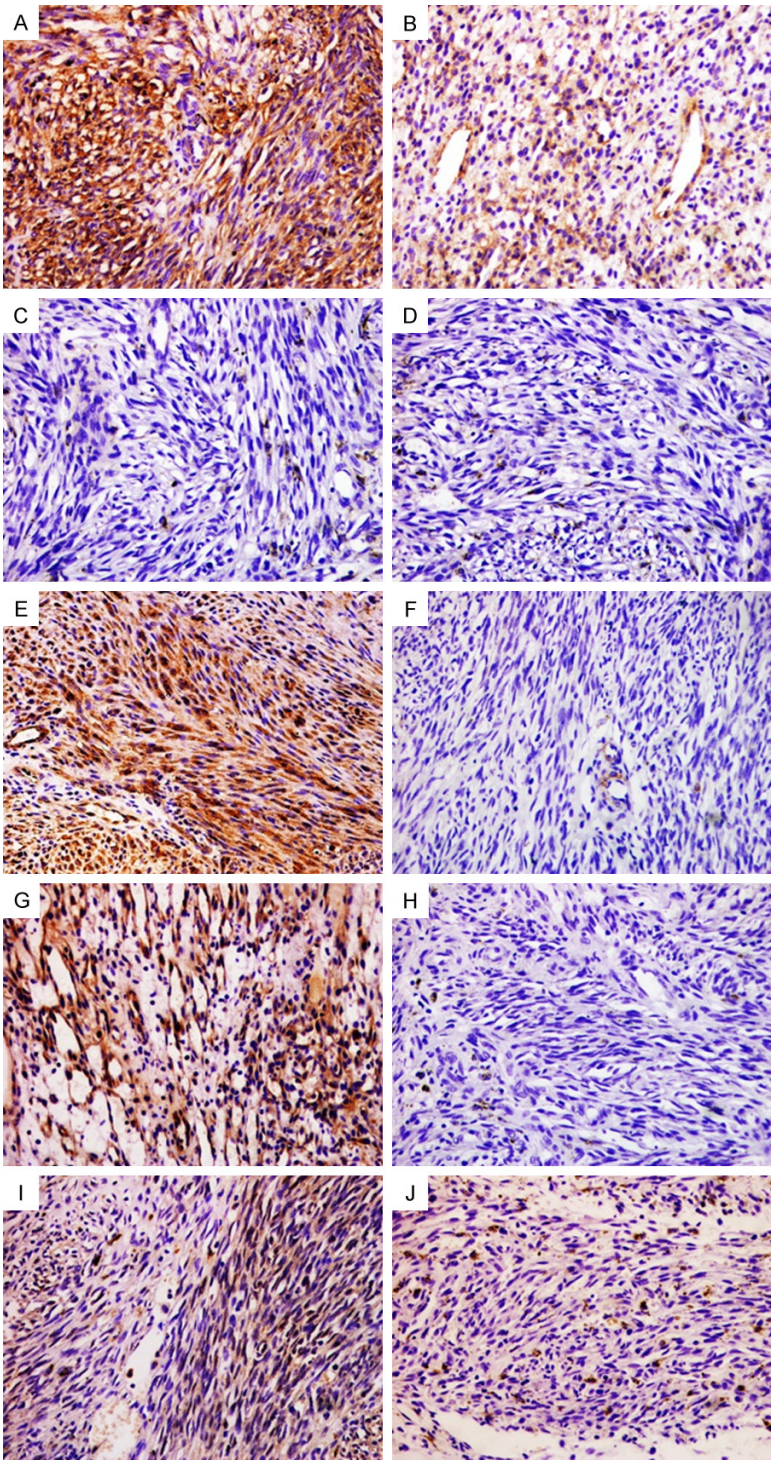

Figure 4. Representative immunohistochemical staining of CD29, CD44, nestin, CD133 and ALDH1 expression in Kaposi's sarcoma tissues. Cytoplasmic positivity staining for CD29 (A and B) was presented and CD44 (C and D) was negative expression in the lesions. Cytoplasmic positivity and corresponding negativity for nestin (E and F) and CD133 (G and H). ALDH1 (I and J) was also negative expression in the lesions. Magnification, ×200.

benign vascular tumors, malignant vascular tumors, and hemangioendothelimas that their clinical behaviors are between the benign hemangiomas and more malignant angiosarcomas. Furthermore, the pathogenesis and histogenesis of different types of vascular tumors are complicated and multi-factorial process [30]. Previous investigations showed that multiple genomic alterations or micro-environmental differences presumably contribute to the development of vascular tumors [7]. Following recent studies supporting the presence of a highly tumorigenic cells subset commonly called cancer stem cells [31-33]. The cancer stem cells hypothesis holds that cancer stem cells may contribute to the initiation, progression and recurrence of cancer [17, 18]. Although the current knowledge of the biological properties of cancer stem cells is very limited, cancer stem cells expressing certain specific surface makers have been documented by several reports. CD133 is a common stem cell surface antigens expressing on hematopoietic stem cells and bone marrow-derived endothelial progenitor cells [34], while CD29, CD44 and nestin have been describe as the mesenchymal stem cells markers [35].

In vascular tumor, cancer stem cells have been regarded as a possible candidate in relation to the origin and pathogenesis of tumors. Khan et al [36] studies firstly reported that the hematopoietic stem cells marker CD133 was used to isolate stem cells from hemangioma. Additionally, CD133 positive tumor cells were observed in the peripheral blood of patients with classic Kaposi's sarcoma [37]. However, Liu et al [11]

Table 7. Comparison of ALDH1, CD133, CD29, CD44 and nestin expression between benign vascular tumors and malignant vascular tumors

Vascular tumors	n	CD29		*P*-value	CD44		*P*-value	Nestin		*P*-value	CD133		*P*-value	ALDH1		*P*-value
		+	-		+	-		+	-		+	-		+	-	
Benign vascular tumors	9	9	0	0.040	3	6	0.319	9	0	0.001	8	1		0	9	
Malignant vascular tumors	30	19	11		4	26		11	19		17	13	0.119	6	24	0.305
Total	39	28	11		7	32		20	19		25	14		6	33	

+, positive; -, negative.

Table 8. Correlation between CD29 and nestin expression in vascular tumors

CD29	Nestin		C value	*P*-value
	Positive	Negative		
Positive	17	3	0.288	0.063
Negative	11	8		
Total	28	11		

confirmed that CD133 detection was negative in almost all cases of hemangiosarcomas and hemangiomas, the hematopoietic stem cells or early endothelial progenitor cells expressing other markers CD34, CD45 and CD117, were participated in tumor formation in vascular tumors. In contrast to previous studies, our study found that most of human hemangiomas and hemangiosarcomas showed strong positive staining for CD133. CD133 is an early marker for hematopoietic stem cells or endothelial progenitor cells, the cellular differentiation or tumor heterogeneity may be responsible for the CD133 expression level [38-40]. In addition to cancer stem cell marker CD133, mesenchymal stem cell markers CD29, CD44 and nestin were observed in vascular tumor. Several studies [41-43] have been reported that the isolated tumor cells from proliferating hemangioma expressed the markers CD29, CD44 and CD105, cell surface marker associated with mesenchymal stem cells. Mesenchymal stem cells are defined by their self-renewal capability and potential for several differentiated cell types [44, 45]. Because of these properties, Yu et al [44] studies demonstrated that mesenchymal stem cells were the source of adipocytes in infantile hemangioma during the involuting and involuted phases. According to our results, CD29 and nestin were positive staining in all cases of hemangiomas, and 3 of 9 hemangioma cases were positive for CD44. In general, the percentage of CD29 and nestin positive tumor cells in hemangiomas was higher than that in hemangiosarcoams. Intermediate filament protein nestin was a new expression marker of mesenchymal stem cells. Nestin is well established cancer stem cell marker for several malignant tumors, such as high malignant glioma [46] and gastrointestinal stromal tumors [47]. Yang et al [48] studies also reported that the expression of nestin was stronger in poorly differentiated hemangiosarcomas compared with well differentiated hemangiosarcomas. These results indicated that vascular tumors were at least partly attribute to the cancer stem cells themselves and that investigations in cancer stem cells may be especially relevant to understanding the pathogenesis of different type vascular tumors.

To our knowledge, ALDH1 have been considered as a marker to identify cancer stem cells derived from human mammary cancer [49], head and neck squamous cell carcinoma [50]. Overexpression of this marker is associated with poor prognosis in breast [49], bladder [51] and lung cancer [52]. However, the role of ALDH1 in vascular tumor progress has not been described previously. In this study, all hemangiomas, Kaposi's sarcomas and hemangioendothelimas were negative for ALDH1 staining, only 5 of 22 hemangiosarcoma cases was positive for ALDH1 with weak to strong staining. In addition, our other groups found (data unpublished) that ALDH1 staining was observed in solitary fibrous tumor (SFT) and perivascular epithelioid cell tumor (PEComa), indicating that the ALDH1 may be a new cancer stem cell marker to explicate the progress of several soft tissue tumors. Thus, further research should be done to identify whether ALDH1 expression may play an important in development and progression of vascular tumors.

In summary, our study showed that five cancer stem cell markers including CD29, CD44, CD133, nestin and ALDH1 exhibited different expression level in different type of vascular tumors. The heterogeneity might be caused by

the different histogenesis and origin of different vascular tumors. According to our results and other previous investigations, we hypothesize that mesenchymal stem cells may contribute to vascular tumor formation. This study provided further insight into the cellular origin leading to formation of vascular, suggesting that cancer stem cells may be involved in vascular tumor characteristics and progression. Therefore, our observation here may need further exploration to investigate the function of cancer stem cells in vascular tumors.

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Disclosure of conflict of interest

None.

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