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Original Article

Clinicopathologic and molecular features of vascular tumors in a series of 118 cases

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Abstract: Aims: Vascular tumors are composed of benign, intermediate, and malignant lesions. The diagnosis is challenging because some entities demonstrate overlapping morphologies and harbor the same genetic alterations. We describe herein a cohort of vascular tumors with clinicopathologic, immunohistochemical, and molecular features. Methods and Results: 118 vascular tumors including 56 angiosarcomas, 18 epithelioid haemangioendotheliomas (EHE), 25 epithelioid haemangiomas (EH), 8 pseudomyogenic haemangioendotheliomas (PHE), 1 papillary intralymphatic angioendothelioma (PILA), 2 kaposiform haemangioendotheliomas (KHE), 3 Kaposi sarcomas, 2 retiform haemangioendotheliomas (RHE), and 3 anastomosing haemangiomas were assessed. FOSB, c-Fos, CAMTA1, and TFE3 expression and gene rearrangements were analyzed by immunohistochemical staining and FISH, respectively. Our results showed that FOSB expression was diffusely positive in all 8 PHEs, focally or sparsely in 12 EHs, and in 2 angiosarcomas. C-FOS expression was sparsely to diffusely positive in 15 EHs, focally or sparsely in 17 angiosarcomas, 1 EHE, 1 Kaposi sarcoma, and 1 PHE. CAMTA1 expression was positive in only 12 EHEs. TFE3 expression was focally or sparsely positive in all 8 PHEs, 22 angiosarcomas, 6 EHEs, 3 EHs, 2 Kaposi sarcomas, and 2 AHs. *FOSB* rearrangement was found in 5 PHEs, *FOS* rearrangement only in 1 EH, *CAMTA1* rearrangement in 4 EHEs. Conclusions: FOSB and CAMTA1 are useful diagnostic markers for PHE and EHE, respectively. *FOSB* and *FOS* fusion represent a subset of epithelioid haemangioma. TFE3 is not a diagnostically meaningful marker in a majority of vascular tumors. The combined utility of these markers will facilitate the differential diagnosis in vascular tumors with morphologic overlap.

Keywords: Vascular tumors, FOSB, c-FOS, CAMTA1, TFE3, fluorescence in situ hybridization

Introduction

Vascular tumors are composed of benign, indeterminate, and low-grade to high-grade malignant ends of the spectrum. The diagnosis is challenging because of morphologic overlap. Angiosarcoma is a group of highly aggressive malignant vascular neoplasms with a predilection for the scalp in elderly men presenting anastomosing vascular spaces lined by noticeably atypical endothelial cells [1]. Epithelioid haemangioendothelioma (EHE) is a low-grade malignant vascular tumor with intracytoplasmic vacuoles in a background of myxohyaline stroma [2]. Kaposi sarcoma is an HHV8-associated low-grade malignant vascular neoplasm and demonstrates slit-like vascular channels shaped by spindled endothelial cells with erythrocyte extravasation [3]. Epithelioid haemangioma (EH) is a benign vascular tumor with well-formed, capillary-sized vessel lumens lined by plump, epithelioid endothelial cells, which predominantly occurs in head and neck [4]. Pseudomyogenic haemangioendothelioma (PHE) is an intermediate vascular tumor with high local recurrence but rarely metastasis [5]. Retiform haemangioendothelioma (RHE), which is an intermediate vascular neoplasm, displays retiform pattern of blood vessels lined by hobnail endothelial cells [6]. Papillary intralymphatic angioendothelioma (PILA, also known as Dabska tumor) is composed of dilated thin-walled blood vessels lined by hobnail endothelium. Focally, hobnail-like endothelial cells show papillary hyperplasia and protrude into vascular lumens like glomeruloid appearance [7]. Kaposiform haemangioendothelioma (KHE) is a locally aggressive vascular tumor and is often

associated with a coagulopathy known as Kasabach-Merritt phenomenon (KMP). Morphologically, KHE displays infiltrating nodules and sheets of spindled endothelial cells surrounded by slit-like vascular channels [8]. Anastomosing haemangioma (AH) is a new entity, which is a rare benign tumor and composed of thin-walled anastomosing vessels lined by a monolayer of endothelial cells with protuberant nuclei [9].

Vascular tumor cells are usually positive for endothelial cell markers such as CD31, CD34, FLI-1, and ERG except that PHE is negative for CD34 [10]. Molecular findings are known in several vascular tumors. Epithelioid haemangioma is characterized by recurrent fusion genes involving the *FOS* or *FOSB* gene in about half of the cases [11, 12]. PHE often harbors *SERPINE-1-FOSB* or *ACTB-FOSB* fusions [13, 14]. EHE is characterized by a t(1;3)(p36;q23-q25) resulting in a *WWTR1-CAMTA1* fusion in more than 90% of cases [15, 16]. In addition, *YAP1-TFE*3 fusion has been identified in a subset of EHE with distinct morphologic features [17].

In this study, we analyzed the clinicopathologic features of 118 cases of vascular tumors with an immunohistochemical panel including FOSB, c-FOS, CAMTA1, and TFE3 and studied genetic features with FISH analysis.

Materials and methods

Patients and specimens

A total of 118 cases of paraffin-embedded vascular tumors specimen were retrieved from Department of Pathology, the First Affiliated Hospital, Sun Yat-sen University between 2010 and 2020. No patients had received chemotherapy and/or radiotherapy before operation. The histopathology of the lesion was determined by two pathologists according to the criteria of the World Health Organization [18]. The clinic information including patients' sex, age, and tumor location was collected. The study was approved by the Institutional Ethics Committee (IEC) for Clinical Research and Animal Trials of the First Affiliated Hospital of Sun Yat-sen University. A request of informed consent waiver was granted by the IEC given the retrospective and minimal-risk nature of the study (No. 2022-103).

Immunohistochemical staining

Immunohistochemistry was performed as we previously described [19]. The working concentration of primary antibodies including FOSB (2251, Cell Signaling Technology, Danvers, MA), c-FOS (TA806833, ZSGB-BIO, Beijing, China), CAMTA1 (NBP1-93620, Novus Biologicals, Littleton, CO), and TFE3 (ZA-0657, ZSGB-BIO, Beijing, China) was 1:800, 1:600, 1:100, and 1:200, respectively. The positive signals of these four antibodies are located in the nuclei of tumor cells.

Fluorescence in situ hybridization (FISH)

FISH was performed on interphase nuclei using dual-color break-apart probes (Guangzhou LBP Medical Technology Co., Ltd., China) specific to the *FOSB*, *FOS*, *CAMTA1*, and *TFE3* gene locus. 3 μM FFPE tissue sections were mounted on slides. The slides were deparaffinized, immersed in xylene and 100% ethanol, air dried, and then microwaved in citric acid. The sections were subsequently pretreated with standard saline citrate, digested with pepsin, and immersed in graded ethanol solutions. Following drying, the probes were applied on each slide, covered with a coverslip, sealed with rubber cement, co-denatured with the target DNA, and then hybridized overnight. After washing, the sections were counterstained with DAPI, and mounted with anti-fade solution. A fused or closely approximated red-green signal pattern was interpreted as a normal result, whereas splitting of the probes indicated the presence of a rearrangement. Gene rearrangement was reported as present if ≥ 10% of the tumor nuclei showed split signals defined as separation of signals.

Results

Clinicopathologic features of 118 vascular tumors

In our series, there were 9 types of vascular tumors including 56 angiosarcomas, 18 epithelioid haemangioendotheliomas, 25 epithelioid haemangiomas, 8 pseudomyogenic haemangioendotheliomas, 2 kaposiform haemangioendotheliomas, 3 Kaposi sarcomas, 2 retiform haemangioendotheliomas, 1 papillary intralymphatic angioendothelioma, and 3 anastomos-

ing haemangiomas. The patients' ages ranged from 2 to 89 years with a median age of 47.5 years. The ratio of males to females was 1.9:1. Tumors were mainly located on head and neck, followed by limbs, bone, and liver. The clinical data is summarized in **Figure 1** and **Table 1**. Briefly, 56 angiosarcomas affected 40 males and 16 females, with a median age of 59.5 years ranging from 26 years to 89 years. The majority of angiosarcomas arose from head and neck, followed by limbs, liver, and bone. 18 EHEs affected 10 males and 8 females with age ranging from 23 to 67 years (median age 47.5 years). Among them, 10 EHEs (10/18, 55.6%) occurred in the liver with multiple lesions, 3 in bone, 2 in lung, 2 in limbs, and 1 in head and neck. 25 epithelioid haemangiomas occurred in 14 males and 11 females with age from 12 years to 65 years (median age 39 years). 13 (13/25, 52%) epithelioid haemangiomas occurred in the head and neck. 8 PHEs were in 6 males and 2 females with age from 7 to 44 years (median age 27.5 years). 5 PHEs occurred in limbs, 2 in bone, 1 in the penis. 2 KHEs included one male, 18 years and one female, 13 years. One KHE patient presented with typical Kasabach-Merritt phenomenon (KMP). All 3 Kaposi sarcomas were in males of age 46 years, 55 years, and 71 years, and all three cases occurred in the foot. None of them were HIV-associated. Two retiform haemangioendotheliomas affected two males of 41 and 36 years respectively. One occurred in a lung and the other one in the trunk. The PILA case affected an 8 years old boy occurred in the limbs. All 3 anastomosing haemangiomas occurred in the kidney and included 2 females and 1 male with age 28, 55, and 67 years, respectively. Tumor cells were positive for CD31, CD34, and ERG. All 3 patients were alive and well after tumor resection after follow-up of 5 years.

FOSB, c-FOS, CAMTA1, and TFE3 expression in vascular tumors by immunohistochemistry

Immunophenotyping results are summarized in **Table 2**. In our study, diffuse and strong nuclear immunoreactivity for FOSB was detected in all 8 PHEs (8/8, 100%) (**Figure 2D**). 12 epithelioid haemangiomas (12/25, 48%) demonstrated focally or sparsely strong nuclear FOSB expression (**Figure 3B**). 2 (3.6%) out of 56 angiosarcomas showed sparse weak FOSB immunoreactivity (**Figure 5D**). However, FOSB expression was not found in other vascular tumors including EHE, KHE, RHE, PILA, Kaposi sarcoma, and AH.

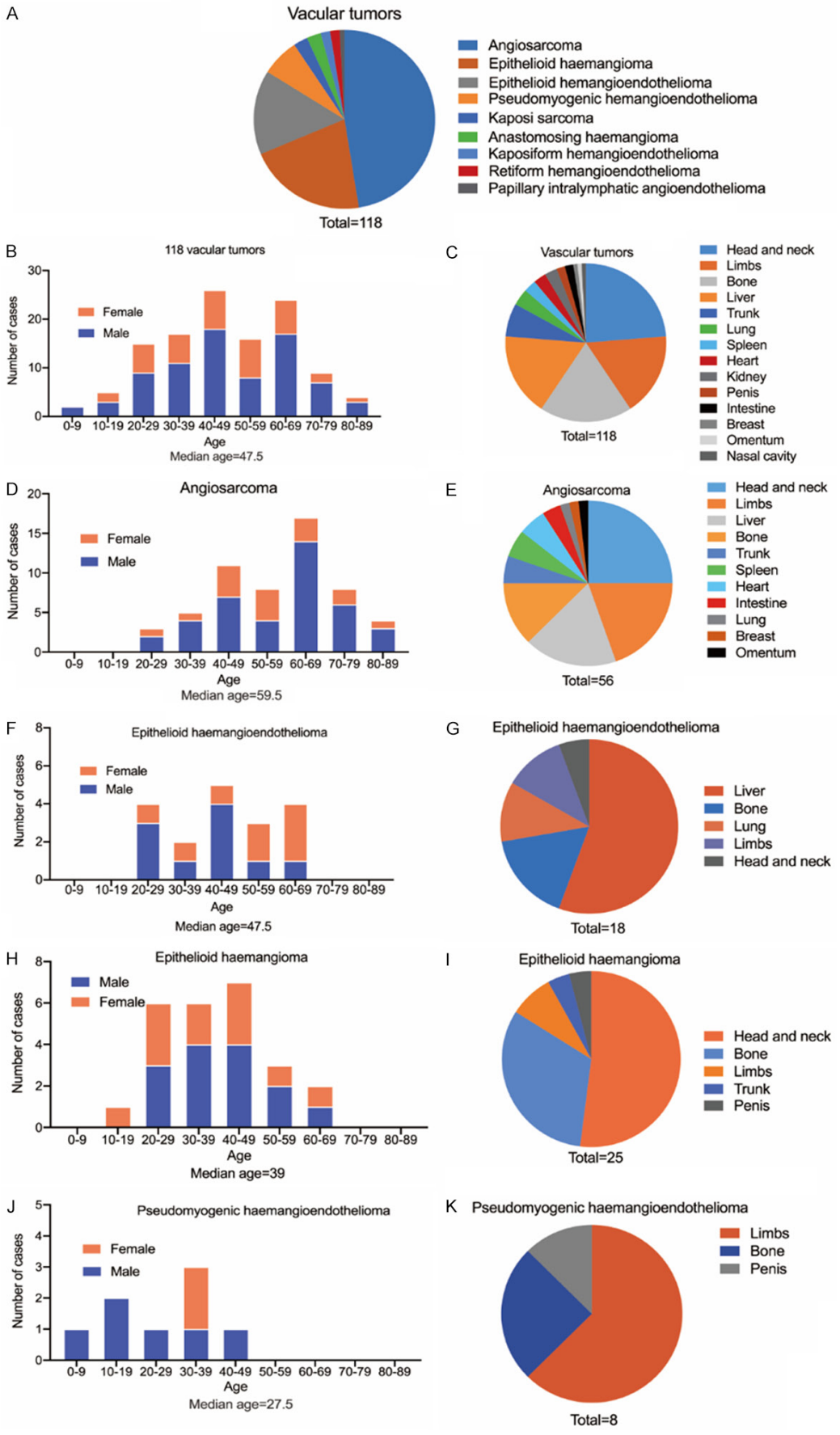
In all 118 vascular tumors, 15 epithelioid haemangiomas (15/25, 60%) were positive for c-FOS staining. Among the 15 c-Fos-positive epithelioid haemangiomas, only one case exhibited strong and diffuse staining for c-FOS (**Figure 3K**), which was an intraosseous cellular epithelioid haemangioma, FOSB-negative. The other 14 epithelioid haemangiomas displayed sparse c-Fos expression (**Figure 3F**). Among 18 EHEs, only one case showed sparsely strong nuclear expression of c-Fos (data not shown). Moreover, 17 angiosarcomas (17/56, 30.6%) exhibited focally moderate or sparsely weak c-Fos immunoreactivity (**Figure 5E**). Furthermore, one PHE and one Kaposi sarcoma demonstrated sparsely strong c-Fos nuclear staining (data not shown). The remaining vascular tumors including KHE (**Figure 6**), RHE, PILA, and AH (**Figure 8**) had no c-Fos expression.

In our series, CAMTA1 expression was extremely limited in EHEs. 12 EHEs (12/18, 66.6%) showed diffuse and strong CAMAT1 positivity (**Figure 4B**, **4E**). However, the other 8 types of vascular tumors had absent CAMTA1 expression.

Diffuse and strong nuclear TFE3 staining was not detected in all 118 vascular tumors. Sparse or focal weak to moderate nuclear TFE3 staining was relatively common in our series. All 8 PHEs showed sparse weak to moderate nuclear expression of TFE3 (**Figure 2E**). 22 angiosarcomas (22/56, 39.3%) displayed diffuse moderate (n=2, **Figure 5F**) or sparse weak (n=20, data not shown) staining of TFE3 but were *TFE3* rearrangement negative (**Figure 5G**). 5 EHEs (5/18, 27.8%) showed sparse weak TFE3 expression (data not shown). 3 epithelioid haemangiomas (3/25, 12%) showed sparse weak TFE3 immunoreactivity (data not shown). 2 out of 3 Kaposi sarcomas (**Figure 7F**) and 2 out of AHs exhibited sparse weak TFE3 staining. TFE3 expression was not found in KHE, PILA, or RHE in our experience.

FOSB, c-FOS, CAMTA1, and TFE3 rearrangement in vascular tumors by FISH

Given that gene overexpression may be driven by gene rearrangement, we next explored


A
Vacular tumors
Angiosarcoma
Epithelioid haemangioma
Epithelioid hemangioendothelioma
Pseudomyogenic hemangioendothelioma
Kaposi sarcoma
Anastomosing haemangioma
Kaposiform hemangioendothelioma
Retiform hemangioendothelioma
Papillary intralymphatic angioendothelioma
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Median age=47.5
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Limbs
Bone
Liver
Trunk
Lung
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Kidney
Penis
Intestine
Breast
Omentum
Nasal cavity
Total=118
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Angiosarcoma
Female
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Number of cases
0-9 10-19 20-29 30-39 40-49 50-59 60-69 70-79 80-89
Age
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Head and neck
Limbs
Liver
Bone
Trunk
Spleen
Heart
Intestine
Lung
Breast
Omentum
Total=56
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Epithelioid haemangioendothelioma
Female
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Number of cases
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Age
Median age=47.5
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Age
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Head and neck
Bone
Limbs
Trunk
Penis
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Pseudomyogenic haemangioendothelioma
Female
Male
Number of cases
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Age
Median age=27.5
K
Pseudomyogenic haemangioendothelioma
Limbs
Bone
Penis
Total=8

Figure 1. Clinical features of 118 vascular tumors. (A) Pie chart shows the percentage of different vascular tumors; (B, C) Age, gender and location distribution of vascular tumors; (D-K) Age, gender and location distribution of angiosarcoma (D, E), epithelioid haemangioendothelioma (F, G), epithelioid haemangioma (H, I), and pseudomyogenic haemangioendothelioma (J, K).

Table 1. Clinical features of vascular tumors in 118 cases

Tumor type	Total cases	Male	Female	Age range (Median) years	Location
Angiosarcoma	56	40	16	26-89 (59.5)	Head and neck (n=14); Limbs (n=11); Liver (n=10); Bone (n=7); Trunk (n=3); Spleen (n=3); Heart (n=3); Intestine (n=2); Lung (n=1); Breast (n=1); Omentum (n=1)
Epithelioid haemangioendothelioma	18	10	8	23-63 (47.5)	Liver (n=10); Bone (n=3); Lung (n=2); Limbs (n=2); Head and neck (n=1)
Epithelioid haemangioma	25	14	11	12-65 (39)	Head and neck (n=13); Bone (n=8); Limbs (n=2); Trunk (n=1); Penis (n=1)
Pseudomyogenic haemangioendothelioma	8	6	2	7-44 (27.5)	Limbs (n=5); Bone (n=2); Penis (n=1)
Kaposiform haemangioendothelioma	2	1	1	13-18 (15.5)	Thoracic spine (n=1); Middle turbinate (n=1)
Kaposi sarcoma	3	3	0	46-77 (55)	Foot (n=3)
Retiform haemangioendothelioma	2	2	0	36-41 (38.5)	Lung (n=1); Trunk (n=1)
Papillary intralymphatic angioendothelioma	1	1	0	8 (8)	Limbs (n=1)
Anastomosing haemangioma	3	1	2	28-67 (55)	Kidney (n=3)

Table 2. Protein expression and FISH detection of FOSB, c-Fos, CAMTA1, and TFE3 in our cases

Tumor type	Total cases	No. (Sensitivity%, Specificity%) of positive expression				No. of gene rearrangement positivity			
		FOSB	c-FOS	CAMTA1	TFE3	*FOSB*	*FOS*	*CAMTA1*	*TFE3*
Angiosarcoma	56	2 (3.6, 67.7)	17 (30.4, 71.0)	0 (0, 80.6)	22 (39.3, 66.1)	ND	ND	ND	0 (0/9)
Epithelioid haemangioendothelioma	18	0 (0, 78.0)	1 (5.6, 66.0)	12 (66.7, 100.0)	6 (33.3, 63.0)	ND	ND	4 (4/9, 44.4%)	ND
Epithelioid haemangioma	25	12 (48.0, 89.2)	15 (60.0, 78.5)	0 (0, 87.1)	3 (12.0, 57.0)	0 (0/3)	1 (1/1, 100%)	ND	ND
Pseudomyogenic haemangioendothelioma	8	8 (100.0, 87.3)	1 (12.5, 69.1)	0 (0, 89.1)	8 (100.0, 68.2)	5 (5/8, 62.5%)	ND	ND	0 (0/2)
Kaposiform haemangioendothelioma	2	0 (0, 81.0)	0 (0, 69.8)	0 (0, 89.7)	0 (0, 63.0)	ND	ND	ND	ND
Kaposi sarcoma	3	0 (0, 80.9)	1 (33.3, 70.4)	0 (0, 89.6)	2 (66.7, 64.3)	ND	ND	ND	ND
Retiform haemangioendothelioma	2	0 (0, 81.0)	0 (0, 69.8)	0 (0, 89.7)	0 (0, 62.9)	ND	ND	ND	ND
Anastomosing haemangioma	3	0 (0, 80.9)	0 (0, 69.6)	0 (0, 89.6)	2 (66.7, 64.3)	ND	ND	ND	ND
Papillary intralymphatic angioendothelioma	1	0 (0, 81.2)	0 (0, 70.1)	0 (0, 89.7)	0 (0, 63.2)	ND	ND	ND	ND

ND: not done.

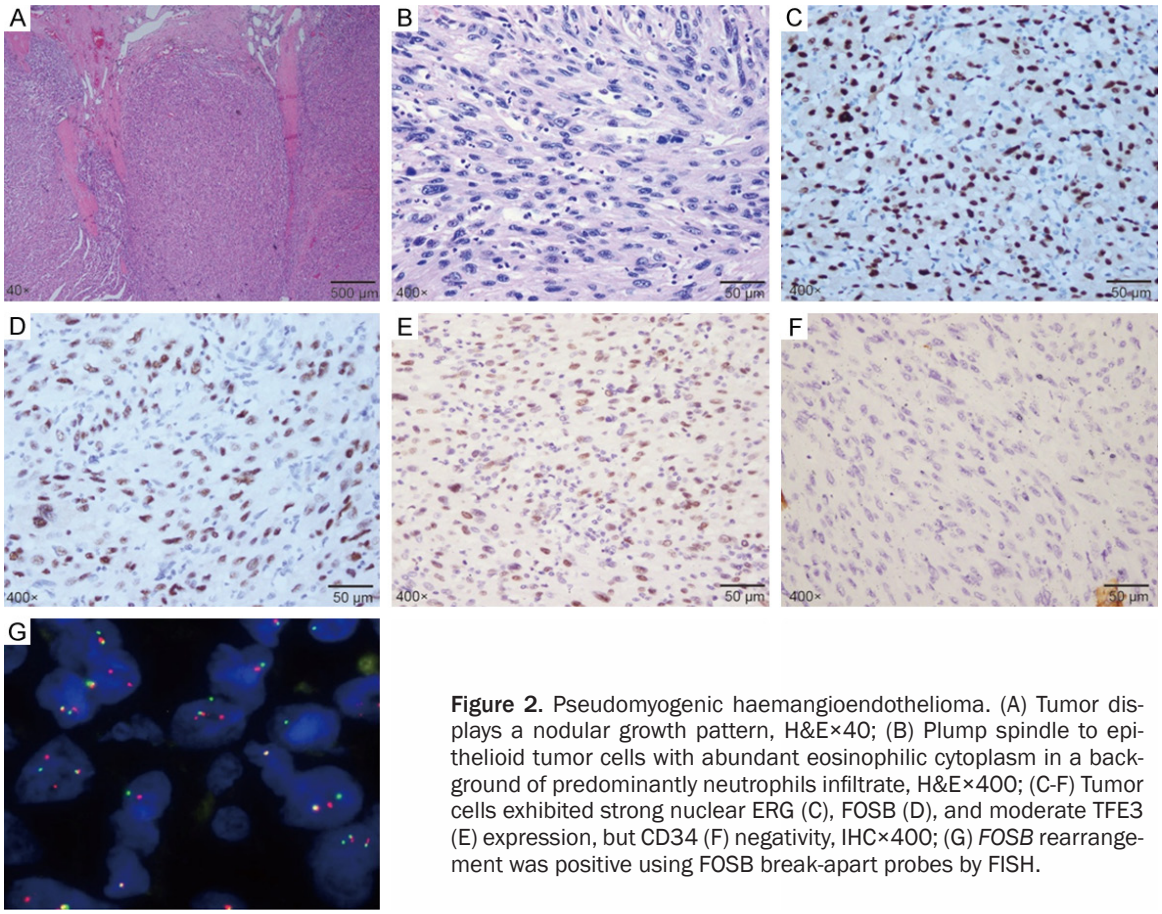

Figure 2. Pseudomyogenic haemangioendothelioma. (A) Tumor displays a nodular growth pattern, H&E×40; (B) Plump spindle to epithelioid tumor cells with abundant eosinophilic cytoplasm in a background of predominantly neutrophils infiltrate, H&E×400; (C-F) Tumor cells exhibited strong nuclear ERG (C), FOSB (D), and moderate TFE3 (E) expression, but CD34 (F) negativity, IHC×400; (G) *FOSB* rearrangement was positive using FOSB break-apart probes by FISH.

whether FOSB, c-FOS, CAMTA1, and TFE3 expression were driven by gene abnormalities in vascular tumors. Our results showed that 5 out of 8 (62.5%) PHE were positive for *FOSB* rearrangements by FISH using break-apart probes (**Figure 2G**). All 12 cases of epithelioid haemangioma with FOSB positivity by immunohistochemical staining were evaluated for *FOSB* rearrangements by FISH. However, 3 cases of epithelioid haemangioma were negative for *FOSB* rearrangement (**Figure 3C**), and the remaining 9 cases of epithelioid haemangioma failed FISH testing due to high background noise. Only one case of intraosseous cellar epithelioid haemangioma with strong c-Fos nuclear staining by immunohistochemistry showed *FOS* gene rearrangement by FISH using break-apart probes (**Figure 3L**). Among 12 CAMTA1-positive EHEs, 4 cases were positive for *CAMTA1* rearrangement (**Figure 4C**), 5 cases were negative for *CAMTA1* rearrangement, and the other 4 cases failed by FISH using break-apart probes. FISH detection for *TFE3* rearrangement was performed in 9 cases of angiosarcoma and 3 cases of PHE with TFE3 focal or sparse expression by immunohistochemistry. All 9 cases of angiosarcoma and 2 cases of PHE were negative for *TFE3* rearrangement. The remaining case of PHE failed for FISH assay (data not shown). The overall genetic features are summarized in **Table 2**.

Discussion

Vascular tumors are composed of a heterogeneous group of entities, which are from benign, indeterminate, and low-grade to high-grade ends of a spectrum. Morphologic similarity among them makes differential diagnosis laborious. A panel of endothelial markers including CD31, CD34, FLI-1, and ERG can help make a diagnosis of vascular tumors, but are of no value in the classification of vascular tumors. Several vascular tumors including EH, PHE, and EHE have been characterized by molecular findings in recent years. *FOS* or *FOSB* gene fusion has been found in about half of epithelioid haemangiomas. *SERPINE-1-FOSB* or *ACTB-*

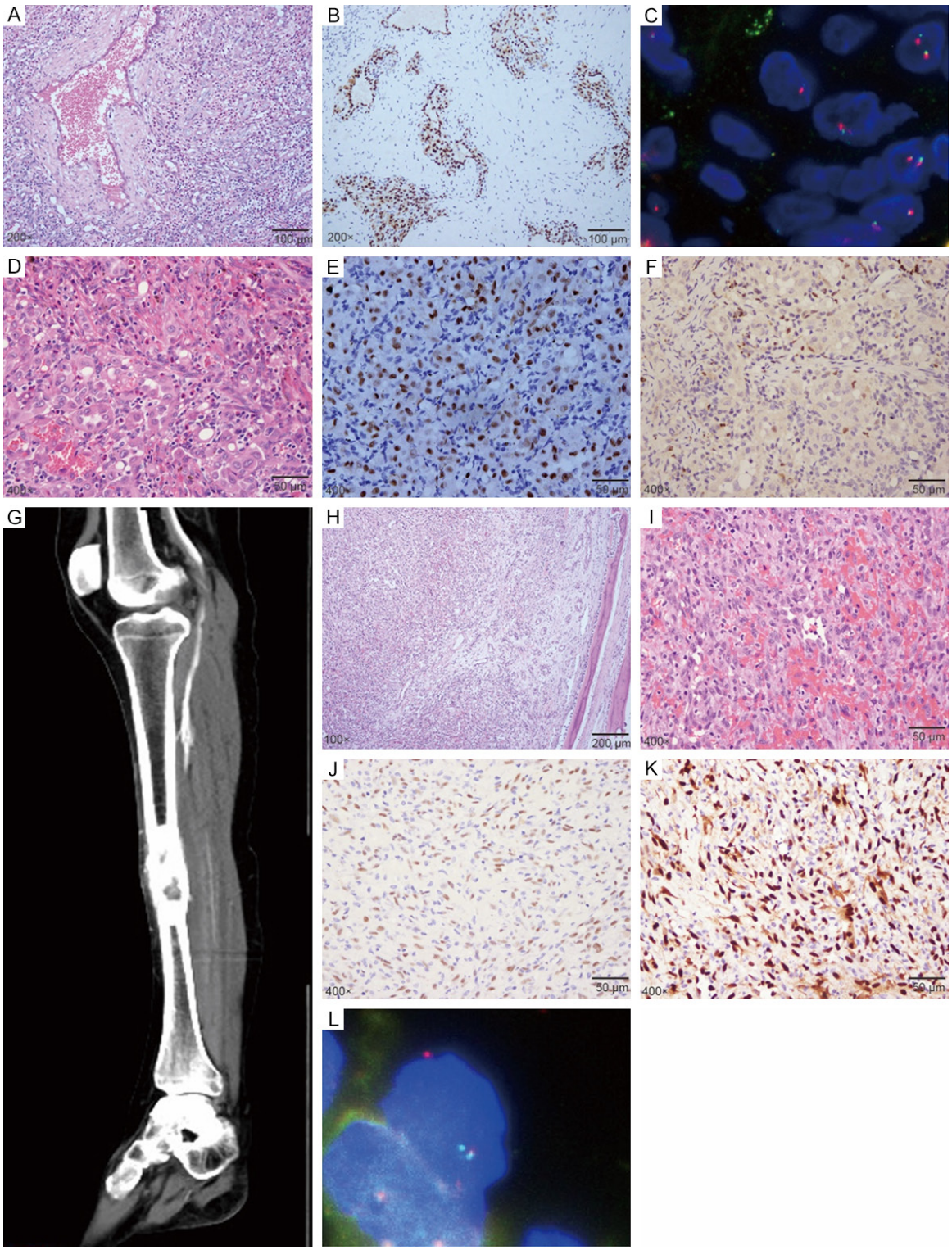

Figure 3. Epithelioid haemangioma. (A) Tumor was composed of well-formed vascular channels lined by plump epithelioid endothelial cells with abundant eosinophilic infiltration in the stroma H&E×200; (B) Tumor cells showed strong nuclear FOSB expression, IHC×200; (C) *FOSB* rearrangement was negative by FISH; (D) Cellular epithelioid haemangioma showed vague nests and sheets of epithelioid endothelial cells with eosinophilic cytoplasm, H&E×400; (E, F) Tumor cells showed diffuse FOSB expression (E) and sparsely weak c-Fos expression (F), IHC×400; (G) CT showed the mass occurred in the middle segment of right tibia, measuring 63×24×22 mm and infiltrated cortical bone; (H) Cellular epithelioid haemangioma displayed solid growth pattern with peripheral vascular forma-

tion and focally infiltrated the bone, H&E×100; (I) Oval or plump spindle tumor cells with abundant eosinophilic cytoplasm and small nucleoli, H&E×400; (J, K) Tumor cells were positive for ERG (J) and c-FOS (K) expression, IHC×400; (L) *c-FOS* break-apart signals were found in tumor cells by FISH.

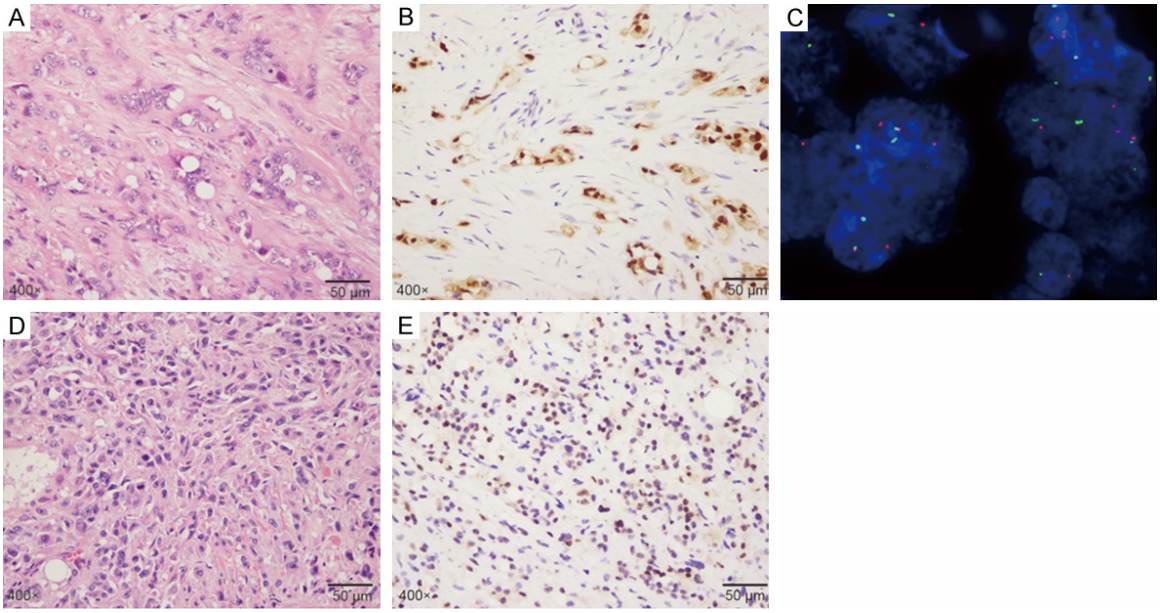

Figure 4. Epithelioid haemangioendothelioma. A. Epithelioid tumor cells with intracytoplasmic vacuoles embedded in a myxohyaline stroma, H&E×400; B. Tumor cells were positive for CAMTA1 expression, IHC×400; C. *CAMTA1* rearrangement was positive in tumor cells by FISH using split-apart probes; D. Epithelioid haemangioendothelioma showed increased cellularity, conspicuous nuclear pleomorphism, and active mitotic figures, H&E×400; E. Strong nuclear CAMTA1 expression was found in tumor cells, IHC×400.

FOSB fusions are common in PHE. *WWTR1-CAMTA1* fusion has been found in more than 90% of EHE. *YAP1-TFE3* fusion has been identified in a subset of EHE with distinct morphologic features. Hence, we evaluated the clinicopathologic features with a panel of markers including FOSB, c-FOS, CAMTA1, and TFE3 and analyzed gene rearrangement in a series of vascular tumors.

In our study, all 8 PHEs showed strong and diffuse nuclear FOSB staining by immunohistochemistry, and 5 PHEs (5/8, 62.5%) harbored *FOSB* rearrangement by FISH, which is compatible with Hung's findings [13]. Besides, 12 epithelioid haemangiomas showed focal or sparse nuclear FOSB expression. We noticed the percentage of FOSB-positive tumor cells in epithelioid haemangioma was much lower than that of PHEs. In 12 epithelioid haemangiomas with FOSB expression, FISH analysis for *FOSB* rearrangement showed 3 epithelioid haemangiomas were negative and 9 cases failed due to high background noise. The results suggest that FOSB expression is common in PHE and epithelioid haemangioma. Additionally, studies have shown that several epithelioid angiosarcomas and epithelioid haemangioendotheliomas exhibited diffuse FOSB expression. Whereas, in our cohort only two angiosarcomas showed sparse and weak nuclear FOSB staining and all 18 EHEs were FOSB-negative, indicating that FOSB expression is not a common event in angiosarcoma and EHE. The translocation t(7;19)(q22;q13), resulting in *SERPINE1-FOSB* gene fusion, is a hallmark genetic abnormality in pseudomyogenic haemangioendothelioma. Several other partner genes of *FOSB* have been identified as well such as *ZNF36* and *WWTR1* in epithelioid haemangioma.

C-Fos, one of the FOS family members encoded by a *FOS* gene, has been described in cementoblastoma and osteoblastoma [20, 21]. *FOS* partner genes include *LMNA* and *VIM* [12]. FOS members can bind Jun proteins and form a dimer, the AP-1 transcription factor complex, to regulate cell processes. In our series, 15 epi-

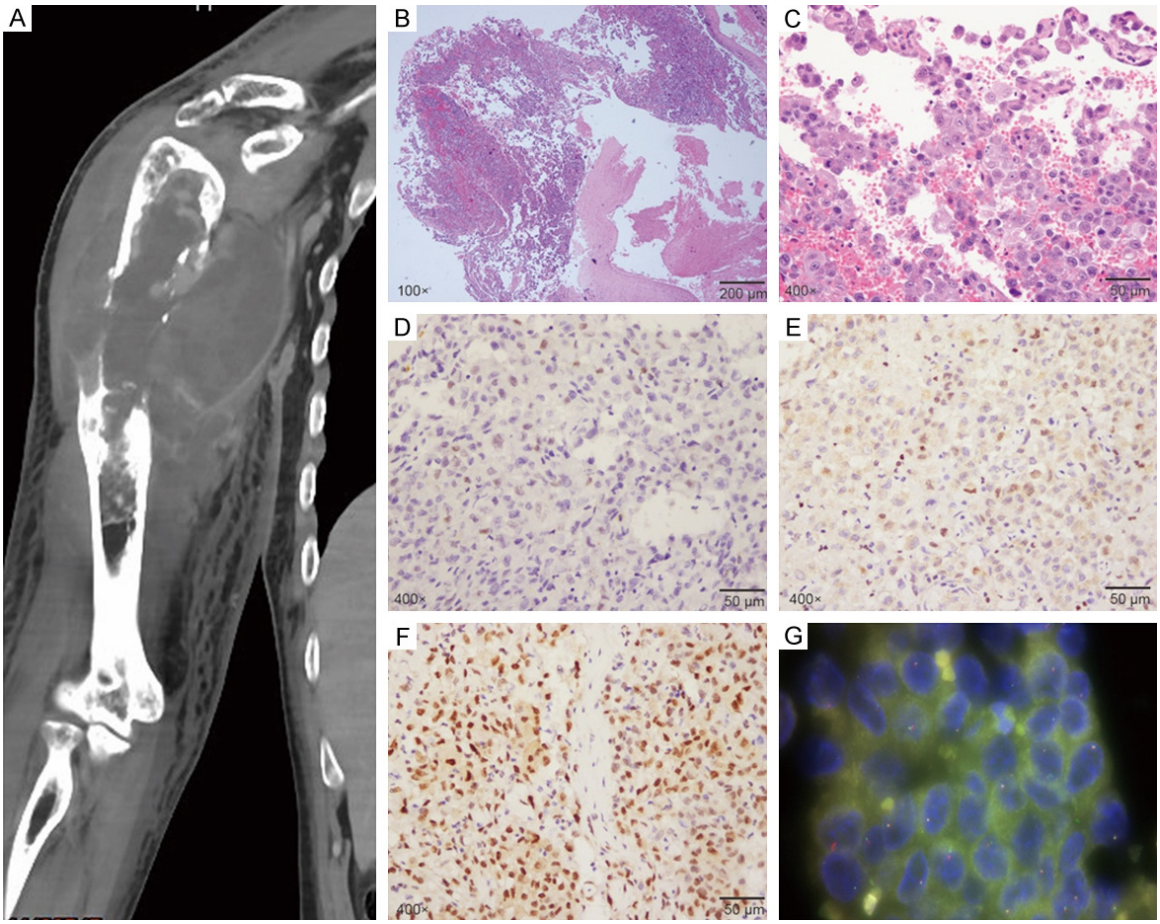

Figure 5. Angiosarcoma. (A) CT scanning demonstrated an ill-defined lesion measuring 135×124×106 mm in right humerus; (B) Tumor presented destructive growth pattern with hemorrhage and necrosis, H&E×100; (C) Tumor was composed of plump epithelioid tumor cells with abundant cytoplasm, nuclear pleomorphism, and brisk mitotic activity, H&E×400; (D, E) Tumor cells displayed weak or moderate FOSB (D) and c-Fos (E) expression, IHC×400; (F) Moderate TFE3 staining was found in tumor cells, IHC×400; (G) FISH analysis revealed *TFE3*-rearrangment was negative.

thelioid haemangiomas (15/25, 60%) were positive for c-FOS staining. Only one intraosseous cellular EH exhibited diffuse, strong c-Fos expression and FISH split probes detected segregated signals indicating *FOS* rearrangement. The remaining 14 cases of epithelioid haemangioma displayed sparse strong c-Fos expression. The lower percentage of *FOS*-fusion in EHs might be related to the prominent inflammatory infiltrates. In addition, other vascular tumors including angiosarcoma, EHE, PHE, and Kaposi sarcoma showed sparsely strong nuclear expression of c-Fos, but the staining pattern is not diagnostically meaningful. *FOS*-fusion represents a molecular feature of a fraction of epithelioid haemangiomas.

Calmodulin binding transcription activator 1 (CAMTA1) normally is highly expressed in brain tissue [22]. 90% EHE cases harbor *WWTR1-CAMTA1* fusion resulting from a recurrent t(1:3)(p36;q23-q25). The fusion enhances CAMTA1 expression and promotes oncogenesis [23]. In our study, CAMTA1 expression was extremely limited in EHEs (12/18, 66.7%). Among 12 CAMTA1-positive EHEs, 4 cases were positive for *CAMTA1* rearrangement by FISH. In our study, the CAMTA1-positive percentage is lower than in the previous study. Actually in our cohort, 12 out of 18 EHE samples were obtained by needle biopsy. Given needle biopsy limitations and shortcomings, we predict the true CAMTA1-positive percentage should be close to 90%. Besides EHEs, CAMTA1 was rarely detected in other tumors. One out of 37 invasive ductal carcinomas of breast displayed CAMTA1 immunoreactivity conducted by *Shibuya*'s study

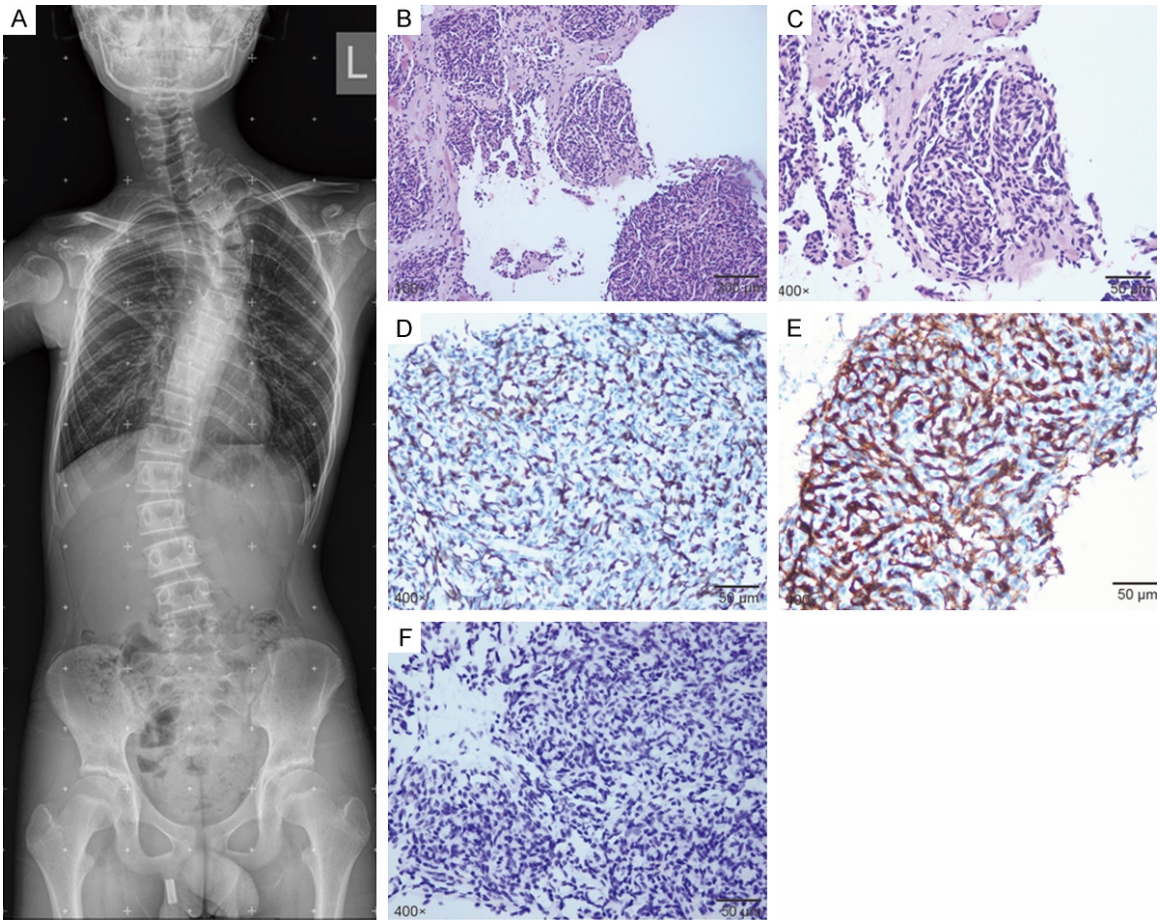

Figure 6. Kaposiform haemangioendothelioma with typical Kasabach-Merritt phenomenon. (A) Digital radiography showed the spine had a typical "S" shape curve in a 13 year old male with scoliosis; (B) Tumor presented a lobular growth pattern, H&E×100; (C) Glomeruloid nodules were composed of spindle endothelial cells with peripheral slit-like vascular channels, H&E×400; (D-F) Tumor cells were positive for ERG (D) and CD34 (E) but negative for HHV8 (F) expression, IHC×400.

[24]. The result indicates that CAMTA1 is a highly sensitive and specific marker for EHE.

TFE3 fusion has been identified in Xp11 translocation renal cell carcinoma [25, 26], alveolar soft part sarcoma [26], and perivascular epithelioid cell tumor [27]. Some tumors, including granular cell tumor [28], pancreatic solid pseudopapillary neoplasm [29], and ovarian sclerosing stromal tumor [30], exhibit TFE3 expression but without *TFE3* fusion. A small subset (<5%) of EHEs harbors TFE3 rearrangement. In our series, none of the 18 EHEs showed diffuse and strong TFE3 immunoreactivity and only 6 EHEs (6/18, 33.3%) showed sparse weak TFE3 expression. The reason might be related to the lower percentage of *TFE3*-rearranged EHEs and too few EHE cases included in the cohort. Sparse or focal weak to moderate nuclear TFE3 staining was also found in other vascular tumors including 8 PHEs, 22 angiosarcomas, 3 epithelioid haemangiomas, 2 Kaposi sarcomas, and 2 anastomosing haemangiomas.

In summary, FOSB and CAMTA1 are useful diagnostic markers to confirm the diagnosis of PHE and EHE, respectively, with high sensitivity and specificity. Besides, *FOSB* and *FOS* fusion represent a subset of epithelioid haemangioma. TFE3 is not a diagnostically meaningful marker in a majority of vascular tumors except *TFE3*-rearranged EHEs. The combined utility of these markers will facilitate the differential diagnosis in vascular tumors with morphologic overlap.

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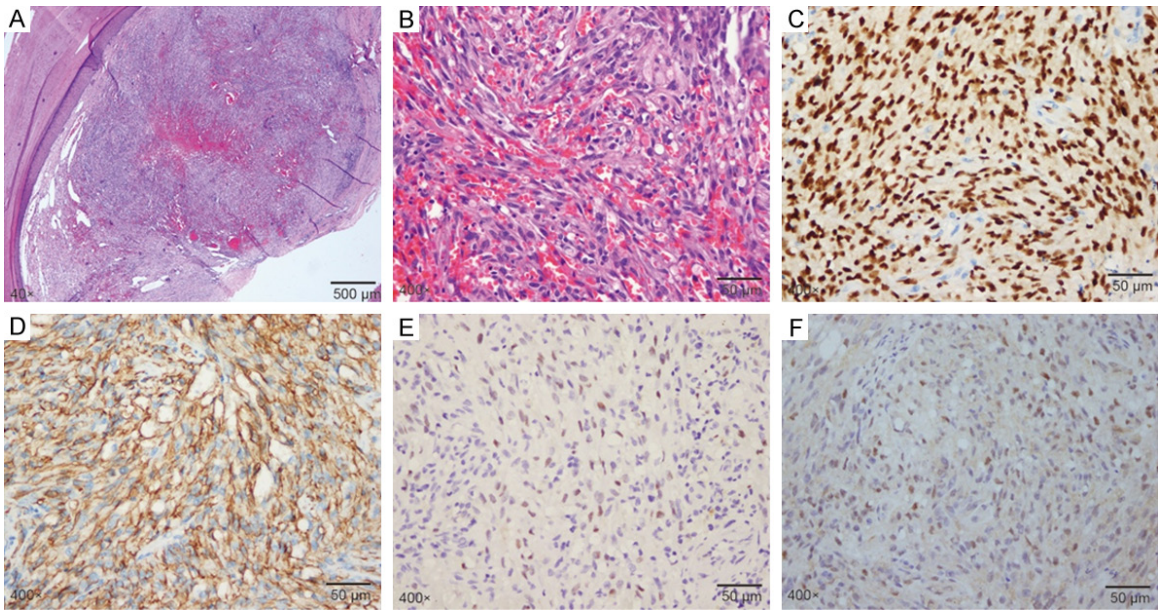

Figure 7. Kaposi sarcoma. (A) A skin nodule lesion demonstrated a well-defined border, H&E×40; (B) Tumor was composed of spindle cells which formed slit blood channels filled with erythrocytes, H&E×400; (C-E) Tumor cells were positive for ERG (C), CD34 (D), and HHV8 (E) expression, IHC×400; (F) Sparse weak TEF3 expression was found in tumor cells, IHC×400.

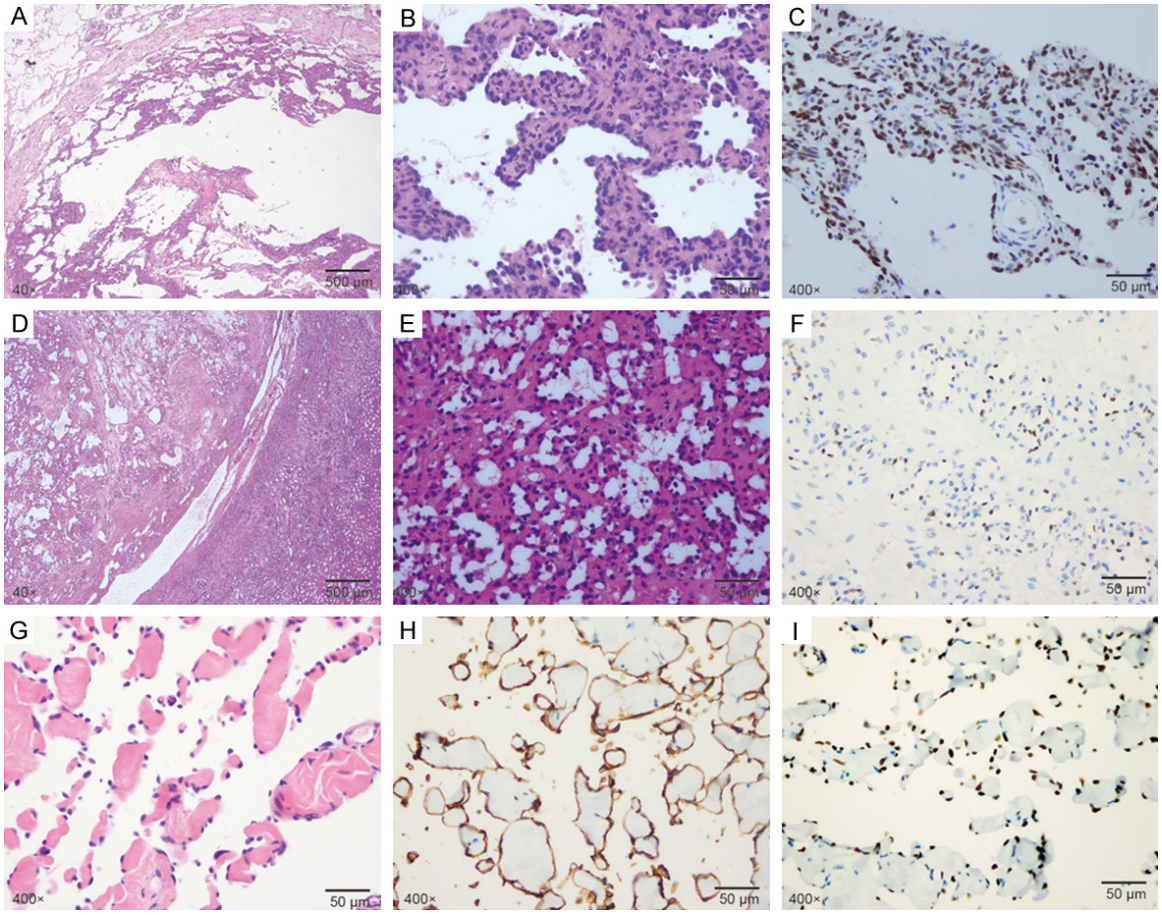

Figure 8. (A-C) Retiform haemangioendothelioma. Tumor was located in the lung and had a clear border, H&E×40 (A); Tumor was composed of retiform blood vessels lined by hobnail endothelial cells with scant cytoplasm, H&E×400 (B); Tumor cells were positive for ERG expression, IHC×400 (C). (D-F) Anastomosing haemangioma. A well-demarcated lesion was found in kidney, H&E×40 (D); Tumor was composed of anastomosing well-differentiated sinusoidal vascular vessels lined by hobnail endothelial cells, IHC×400 (E); ERG expression was found in tumor cells, IHC×400 (F). (G-I) Papillary intralymphatic angioendothelioma. Tumor was composed of dilated vessels lining histiocytic-like endothelial cells, H&E×400 (G); Tumor cells were positive for D2-40 (H) and ERG (I) expression, IHC×400.

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Disclosure of conflict of interest

None.

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