Original Article
Clinicopathological and cytogenetic features of hepatic angiosarcoma and epithelioid hemangioendothelioma

Zhan-Bo Wang*, Jin-Hong Liu*, Jing Yuan, Huai-Yin Shi

*Department of Pathology, Chinese People's Liberation Army General Hospital, Beijing, China. *Equal contributors and co-first authors.*

Abstract: Background and Objectives: Hepatic angiosarcoma (HAS) and epithelioid hemangioendothelioma (HEHE), are rare liver malignancies. This study aimed to evaluate prognostic factors, including erythroblast transformation-specific-related gene (ERG) expression, in HAS and HEHE. Methods: Twenty-four HAS patients and 38 HEHE patients were retrospectively enrolled between January 1986 and June 2014. Immunohistochemistry was performed to examine expression of CD31, CD34, factor VIII-related antigen and ERG, and fluorescence *in situ* hybridization was used to determine expression and rearrangement of *ERG*. Results: HAS patients had a significantly shorter mean overall survival than HEHE patients ($P < 0.05$). Multivariate analysis showed that presence of extrahepatic metastases ($P = 0.04$) and histological necrosis ($P = 0.02$) were independent unfavorable prognostic factors for HAS, and presence of extrahepatic metastases ($P < 0.01$), advanced mitosis grading ($P < 0.01$), and high Ki-67 proliferation index ($P < 0.01$) were independent unfavorable prognostic factors for HEHE. *ERG* expression without any gene rearrangement was identified in HAS and HEHE. Conclusions: Presence of extrahepatic metastases and advanced mitosis grading are independent unfavorable prognostic factors for HAS and HEHE, with ERG protein as a highly sensitive biomarker.

Keywords: Hepatic angiosarcoma, hepatic epithelioid hemangioendothelioma, clinicopathology, prognostic factor, erythroblast transformation-specific-related gene, gene rearrangement

Introduction

Primary malignant vascular tumors, such as hepatic angiosarcoma (HAS) and epithelioid hemangioendothelioma (HEHE), are rare liver malignancies. The clinicopathological features of HAS and HEHE are not well documented in the current literature due to the rarity of these two types of liver vascular tumors. HAS is the most common primary liver sarcoma associated with environmental or occupational exposure to carcinogens [1]. HEHE is a rare malignant liver tumor of low-to-moderate malignancy with a natural history between hemangioma and angiosarcoma [2]. A diagnostic challenge facing surgical oncologists and clinical pathologists encountering HAS and HEHE is that these two pathological entities have no specific clinical manifestations but instead confounding histological characteristics, leading to a high risk of misdiagnosis and missed diagnosis.

Multiple vascular endothelial markers, such as CD31, CD34, and factor VIII-related antigen

(FVIIIRAG) [3], are used for the pathological diagnosis of HAS and HEHE but have a limited role due to low sensitivity and specificity. Erythroblast transformation-specific-related gene (ERG) is a member of the erythroblast transformation-specific family of transcription factors [3, 4]. The *ERG* gene encodes a transcriptional regulator, which functions to regulate angiogenesis and endothelial cell apoptosis [4, 5]. The *ERG* gene is expressed by endothelial cells but not by other vascular stromal cells or myoepithelial cells [3, 4]. Thus, the *ERG* gene is undetectable in the majority of carcinomas and sarcomas except for occasional expression in prostate cancer, epithelioid sarcoma, and Ewing sarcoma [4, 5]. In contrast, ERG is known to be expressed in almost 100% of HAS cases [5, 6], and its expression remains unknown in HEHE patients. Moreover, gene fusion, a potential etiology, was reported in ERG-positive prostate cancer (*TMPRSS2-ERG*) and Ewing sarcoma patients (*TMPRSS2-ERG*) [3, 4] and has yet to be investigated in HAS and

Table 1. Antibodies for Immunohistochemistry

Antibodies	Clone ID	Vendor	Working dilution	Location of positivity
Vimentin	V9	Invitrogen	1:200	Cytoplasm
Ki67	MIB-1	DAKO	1:100	Cellular nucleus
CD31	Jc70A	GeneTech	1:100	Cell membrane
CD34	QBEnd-10	DAKO	1:50	Cell membrane
FVIII	FVIIIRAG/86	DAKO	1:50	Cytoplasm
ERG	Ep111	Epitomics	1:100	Cellular nucleus

Table 2. Clinical Characteristics of HAS Patients and HEHE Patients

	HAS (n = 24)	HEHE (n = 38)
Age, year, mean ± SD (range)	50.6 ± 19.8 (7-86)	46.2 ± 13.2 (26-71)
Sex, male:female	14:10	20:18
Clinical presentations, n (%)		
Abdominal pain	16 (66.7)	25 (65.8)
Fever	0 (0.0)	1 (2.6)
Loss of body weight	0 (0.0)	3 (7.9)
Jaundice	0 (0.0)	1 (2.6)
Back pain	3 (12.5)	0 (0.0)
Palpitation	1 (4.2)	0 (0.0)
Cough	1 (4.2)	1 (2.6)
Asymptomatic	3 (12.5)	8 (21.1)
Laboratory abnormality, n (%)		
HBsAg positivity	2 (8.3)	2 (5.3)
Tumor size, cm, mean ± SD (range)	9.6 ± 6.6 (3-27)	4.6 ± 2.7 (1-10)
Number of liver disease, n (%)		
Single	20 (83.3)	11 (28.9)
Multiple	4 (16.7)	27 (71.1)
Location of liver disease, n (%)		
Left lobe	6 (25.0)	8 (21.1)
Right lobe	15 (62.5)	22 (57.9)
Bilateral lobes	3 (12.5)	8 (21.1)
Presence of extrahepatic metastases, n (%)	7 (29.2)	10 (26.3)
Treatment modalities, n (%)		
Surgical resection	11 (45.8)	9 (23.7)
Liver transplantation	2 (8.3)	0 (0.0)
Interventional therapy	8 (33.3)	26 (68.4)
Medical treatment alone	3 (12.5)	2 (5.3)

HEHE patients. The primary objective of this study was to evaluate the clinicopathological features and *ERG* gene expression profile in HAS and HEHE.

Materials and methods

Study protocol

Twenty-four HAS patients and 38 HEHE patients were retrospectively and consecutively referred to the Department of Clinical Pathology for definitive diagnosis between January 1986 and June 2014. All patients or legal representatives volunteered to give informed consent prior to pathological examination. Pathological examinations were performed on specimens obtained by surgical resection (13 HAS, 9 HEHE), liver needle aspiration biopsy (8 HAS, 29 HEHE), and autopsy (3 HAS, 0 HEHE). Clinical characteristics, such as age, sex, clinical manifestations,

Table 3. Histological Characteristics of HAS and HEHE

	HAS (n = 24)	HEHE (n = 38)
Presence of tumor necrosis, n (%)	12 (50.0)	20 (52.6)
Mitosis grading, n (%)		
Grade 1	1 (4.2)	21 (55.3)
Grade 2	3 (12.5)	6 (15.8)
Grade 3	5 (20.8)	6 (15.8)
Grade 4	9 (37.5)	4 (10.5)
Grade 5	6 (25.0)	1 (2.6)
Ki67 proliferation index, n (%)		
Grade 1	0 (0.0)	22 (57.9)
Grade 2	0 (0.0)	6 (15.8)
Grade 3	6 (25.0)	4 (10.5)
Grade 4	3 (12.5)	0 (0.0)
Grade 5	15 (62.5)	6 (15.8)
Immunohistochemical positivity, n (%)		
Vimentin	18(75.0)	31 (81.5)
CD31	19 (79.1)	32 (84.2)
CD34	21 (87.5)	33 (86.8)
FVIIIRAG	10 (41.7)	16 (42.1)
ERG	24 (100.0)	38 (100.0)

laboratory examinations, and treatment course, were retrospectively collected by reviewing the patients' medical charts. All patients were followed by an independent research staff using a standardized telephone survey questionnaire by November 2013.

Pathologic examination

Specimens were fixed in 4% formalin and embedded in paraffin according to routine procedures, and light microscopy (Olympus, Tokyo, Japan) with hematoxylin-eosin staining was performed to examine the presence of cellular atypia and histological necrosis. The number of mitotic cells was counted under 50 high-power fields (HPFs; 0.24 mm^2) at a magnification of 400×. The mean number of mitotic cells per 10 HPFs was used for mitosis grading as follows: grade 1, 0; grade 2, 1-2; grade 3, 3-4; grade 4, 5-9; and grade 5, ≥ 10.

Immunohistochemistry

Immunohistochemistry was performed using the EnVision kit (DAKO, Glostrup, Denmark) with the antibodies listed in **Table 1**. The Ki67 proliferation index was calculated as the number of positive cells per 2,000 tumor cells, and the percentage was graded as follows: grade 1,

0-4%; grade 2, 5-9%; grade 3, 10-14%; grade 4, 15-19%; and grade 5, > 20%.

ERG fluorescence in situ hybridization

Fluorescence *in situ* hybridization (FISH) was performed using the ZytoLight SPEC ERG Dual Color Break Apart Probe (ZytoVision GmbH, Bremerhaven, Germany) to examine *ERG* gene transcription and rearrangement. At least 100 tumor cell nuclei were scored for each specimen. A cutoff value of 10% was used for the assessment of the *ERG* gene rearrangement FISH results, as previously reported by Miettinen et al. [7].

Statistical analysis

All experiments were performed in duplicate and independently repeated in triplicate. The statistical software package SPSS 17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. All continuous data were expressed as mean ± standard deviation, and all categorical data were expressed as n (%). Quantitative variables were compared using the Mann-Whitney U-test. Proportions were compared using the chi-square test or Fisher's exact test. Survival estimates were calculated using the Kaplan-Meier method and compared using the log-rank test. The Cox regression model was used for multivariate analysis. A two-tailed *P*-value < 0.05 was considered statistically significant.

Results

Clinical data

The clinical characteristics of HAS patients and HEHE patients are described in **Table 2**. These two malignancies mainly affected middle-aged men and women without an obvious sex predominance. Abdominal pain was the most frequent chief complaint for HAS patients (66.7%) and for HEHE patients (65.8%), while a minority of patients were asymptomatic (12.5% for HAS patients and 21.1% for HEHE patients). Fever, loss of body weight, and jaundice were occasionally seen in HEHE patients. Hepatitis B surface antigen positivity was observed in 8.3% of
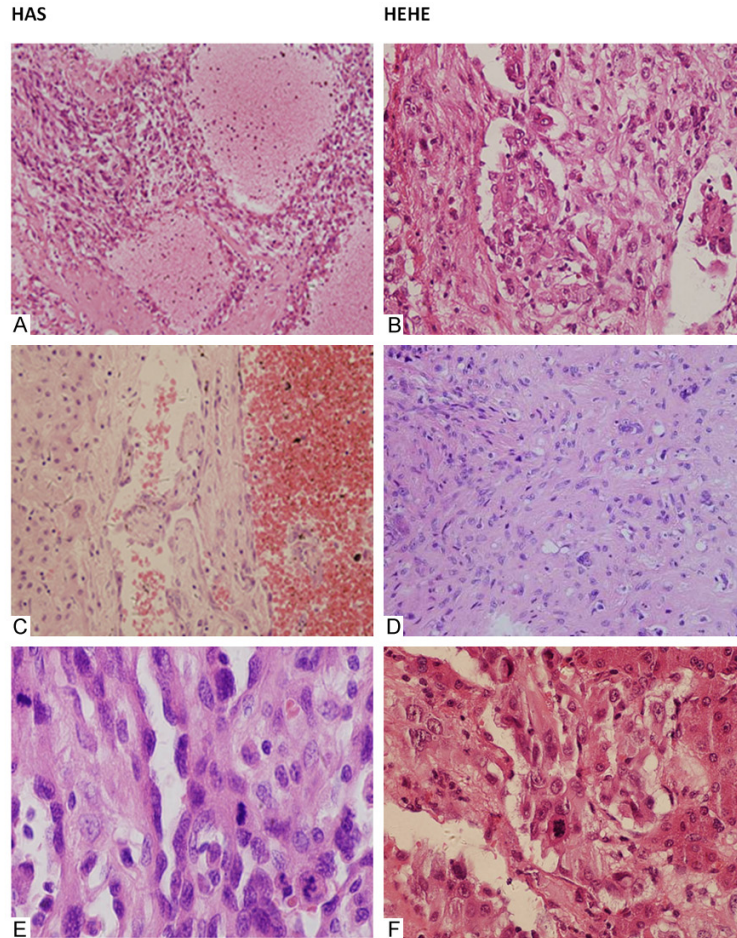
Figure 1. Histology (hematoxylin-eosin) of HAS and HEHE: HAS manifests as (A) an enlarged vascular lumen (200×) lined by (C) overproliferated, atypical, papillary-like endothelial cells (400×), with (E) frequent atypical cells and mitoses (400×); HEHE manifests as (B) polyp-like hyperplasia of epithelioid cells in the blood sinus (200×) and intermixed with (D) dendritic-like cells in a short spindle shape and with eosinophilic cytoplasm and occasional vacuoles (400×), and (F) frequent mitoses are seen in occasional cases (400×).

HAS had abundant vascular tissue (**Figure 1A**), including cavernous hemangioma-like structures (n = 2), enlarged blood sinus (n = 6), and erythrocyte-filled vessels (n = 2). The neoplastic vessels were lined by mono- or multi-layered atypical tumor cells (**Figure 1C**). Parenchymal cells were mainly composed of hyperpla-stic nodular spindle-shaped cells with frequent mitoses. HEHE normally presented as a grayish nodular, rigid mass, with relatively less histological destruction caused by tumor infiltration and intermixing of the cellular component with mucus-like stromal component (**Figure 1B**). Tumor cells mainly consisted of epithelioid, dendritic-like, and intermediate-type cells with occasional mitoses (**Figure 1D**). Advanced mitosis grading (grade 3-5) was seen in 83.3% of HAS patients (**Figure 1E**) and 28.9% of HEHE patients (**Figure 1F**).

Consistently, HAS exhibited a higher proliferation index (grade 3-5, 100%) compared to HEHE (26.3%) as shown by Ki67 immunohistochemistry (**Figure 2A** and **2B**). Approximately 80% of both HAS and HEHE patients were immunopositive for CD31 (**Figure 2C** and **2D**) and CD34 (**Figure 2E** and **2F**), while FVIIIRAG staining was positive in approximately 40% of patients. All HAS and HEHE patients were positive for *ERG* expression (**Figure 2G** and **2H**), and *ERG* expression was mainly located in cell nuclei for HAS and mainly in cell nuclei and occasionally in cytoplasm for HEHE. Among 16 HAS specimens and 9 HEHE specimens sampled for *ERG* gene expression via FISH, no ERG gene rearrangement signal was detected (**Figure 3**).

HAS patients and 5.3% of HEHE patients. HAS mainly presented as a single liver disease (83.3%), whereas HEHE frequently manifested as multiple diseases (71.1%). The right lobe was more frequently involved than the left counterpart, and 12.5% of HAS patients and 21.1% of HEHE patients had bilateral lobe involvement. Extrahepatic metastases were present in 29.2% of HAS patients and 26.3% of HEHE patients at the time of diagnosis. The primary treatment modality was surgical resection for HAS (45.8%) and interventional therapy for HEHE (68.4%).

Histologic and immunohistochemical data

The histological and immunohistochemical features are described in **Table 3**.

Survival data

Overall, all but 8 patients were followed up for a mean period of 60 months until the last visit by
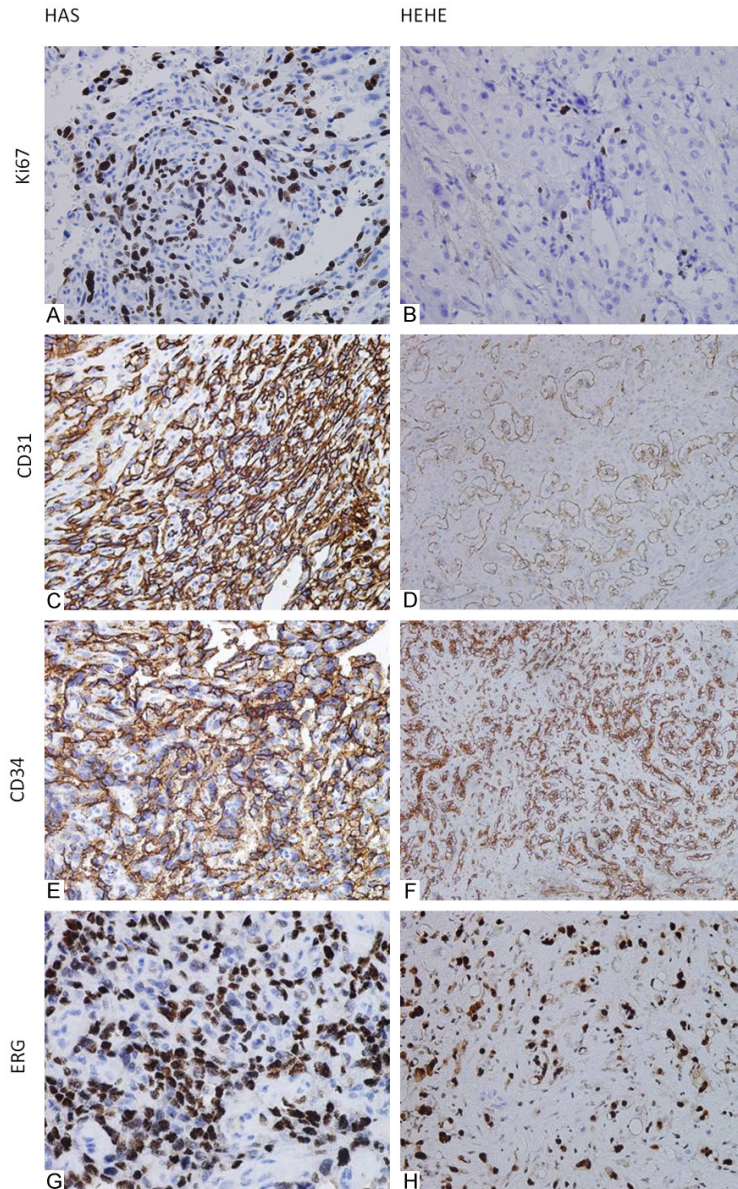
Figure 2. Immunohistochemistry (200×) of HAS and HEHE: (A) and (B) Ki67-immunopositive cell nuclei; (C) and (D) CD31-immunopositive cell membranes; (E) and (F) CD34-immunopositive cell membranes; and (G) ERG-immunopositive nuclei in HAS and (H) positive nuclei and occasional cytoplasm in HEHE. Cell nuclei are counterstained with hematoxylin.

Prognostic factors

Among HAS patients, men and women had a similar overall survival (11.7 ± 3.4 mo. *vs.* 14.9 ± 4.8 mo., *P* > 0.05); those with multiple liver diseases had a significantly longer survival than those with a single disease (23.3 ± 12.3 mo. *vs.* 11.6 ± 2.7 mo., *P* < 0.05). However, the presence of extrahepatic metastases (5.7 ± 1.4 mo. *vs.* 15.6 ± 3.7 mo., *P* < 0.05; **Figure 4B**), advanced mitosis grading (grade 3 *vs* grade 1/2, 13.4 ± 4.5 mo. *vs.* 34.0 ± 0.0 mo., *P* < 0.05), and higher Ki67 proliferation index (grade 5 *vs.* grade 4 *vs.* grade 3, 10.3 ± 3.4 mo. *vs.* 10.0 ± 1.2 mo. *vs.* 23.0 ± 6.2 mo., *P* < 0.01) were associated with a significantly shorter overall survival.

Among HEHE patients, men and women had a similar overall survival (23.7 ± 3.5 mo. *vs.* 34.4 ± 6.9 mo., *P* > 0.05); those with multiple liver diseases also had a similar survival to those with a single disease (29.2 ± 6.2 mo. *vs.* 28.1 ± 3.9 mo., *P* > 0.05). However, the presence of extrahepatic metastases (14.6 ± 5.3 mo. *vs.* 40.0 ± 3.6 mo., *P* < 0.05; **Figure 4C**), advanced mitosis grading (grade 4 *vs.* grade 3 *vs.* grade 2 *vs.* grade 1, 5.2 ± 2.0 mo. *vs.* 9.8 ± 1.2 mo. *vs.* 18.4 ± 2.5 mo. *vs.* 60.0 ± 0.0 mo., *P* < 0.05), and higher Ki67 proliferation index (grade 5 *vs.* grade 3 *vs.* grade 2 *vs.* grade 1, 7.2 ± 2.2 mo. *vs.* 11.0 ± 2.0 mo. *vs.* 19.0 ± 3.5 mo. *vs.* 53.3 ± 5.5 mo., *P* < 0.01) were associated with a significantly shorter overall survival.

Univariate and multivariate analyses

Univariate and multivariate analyses for potential prognostic factors of HAS and HEHE are shown in **Table 4**. Univariate analysis showed that the presence of a single disease (*P* <
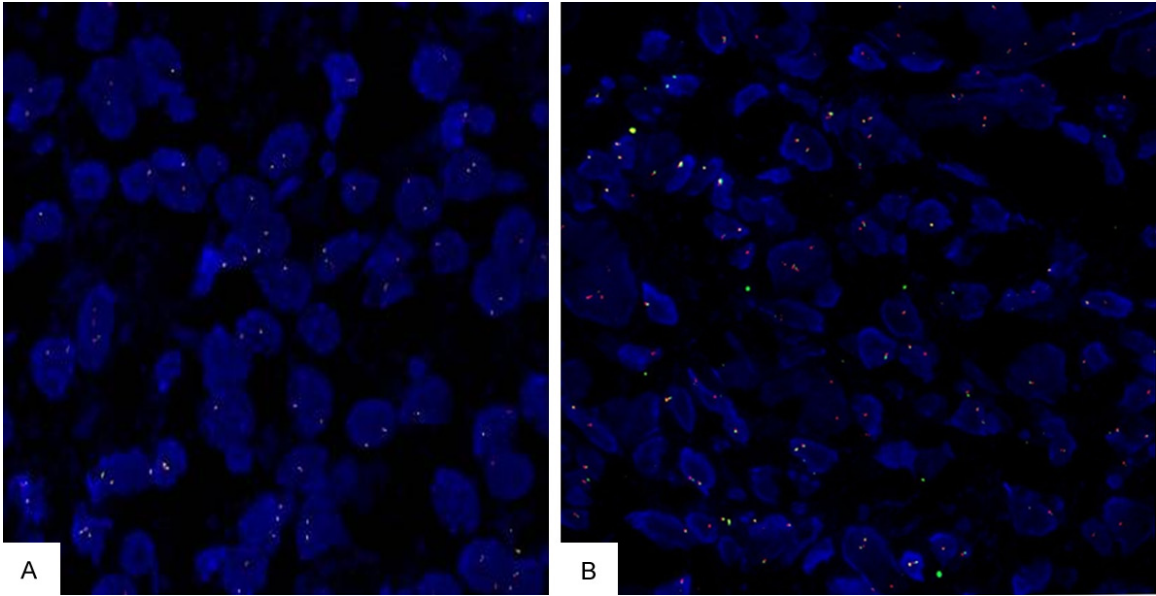
December 2013. Three HAS patients underwent autopsy only, and 18 HAS patients died of disease progression and HAS-associated morbidities. Seventeen HEHE patients survived, with 5 patients lost to follow-up and 16 patients dying of disease progression and HEHE-associated morbidities. The mean overall survival was 12.2 ± 2.7 months for HAS patients and 34.9 ± 4.9 months for HEHE patients (**Figure 4A**).

Figure 3. Fluorescence *in situ* hybridization of *ERG* gene: negative signal of *ERG* gene rearrangement in (A) HAS and (B) HEHE.
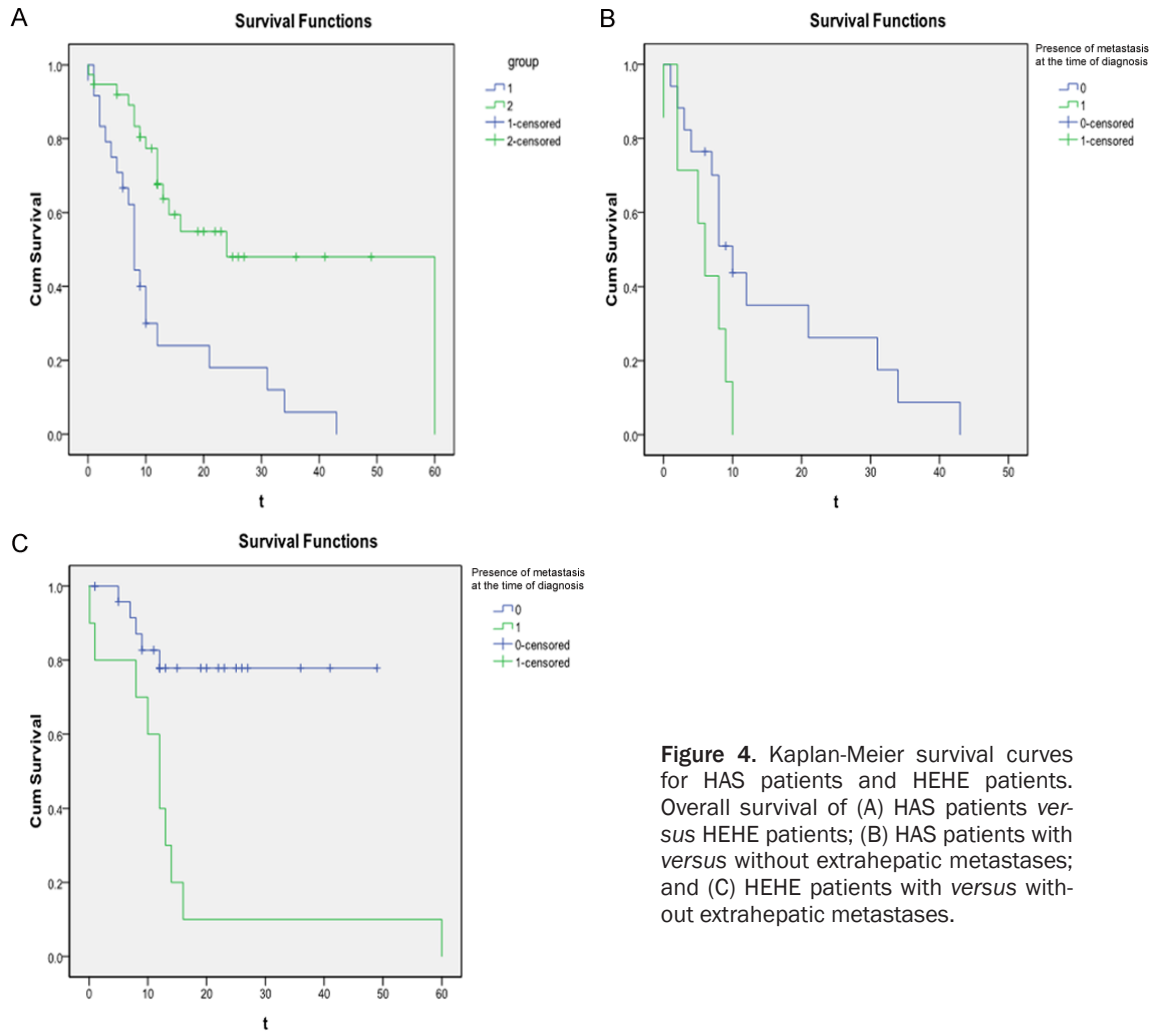

Figure 4. Kaplan-Meier survival curves for HAS patients and HEHE patients. Overall survival of (A) HAS patients *versus* HEHE patients; (B) HAS patients with *versus* without extrahepatic metastases; and (C) HEHE patients with *versus* without extrahepatic metastases.

Table 4. Univariate and Multivariate Analyses of Potential Prognostic Factors for HAS and HEHE

	HAS patients (n = 24)					HEHE patients (n = 38)			
	n	Mean OS (months)	P-value Univariate	P-value Multivariate		n	Mean OS (months)	P-value Univariate	P-value Multivariate
Age, year			0.632	N/A				0.915	N/A
< 50	10	15.0			< 46 20		34.2		
≥ 50	14	11.8			≥ 46 18		29.9		
Sex			0.972	N/A				0.923	N/A
Male	14	11.7				20	23.7		
Female	10	14.9				18	34.4		
Tumor size, cm			0.575	N/A				0.076	N/A
< 9.6	15	15.0			< 4.6 21		31.4		
≥ 9.6	9	10.3			≥ 4.6 17		11.0		
Number of liver disease			< 0.001	0.292				0.176	N/A
Single	20	11.6				11	28.1		
Multiple	4	23.3				27	29.2		
Presence of extrahepatic metastases			0.035	0.043				0.001	0.104
Yes	7	5.7				10	14.6		
No	17	15.6				28	40.0		
Histological necrosis			0.067	N/A				0.029	0.880
Yes	12	16.9				18	26.0		
No	12	8.1				20	40.6		
Mitosis grading			0.016	0.018				< 0.001	< 0.001
Grade 1	1	9.0				21	60.0		
Grade 2	3	34.0				6	18.4		
Grade 3	5	13.4				6	9.8		
Grade 4	9	5.1				5	5.2		
Grade 5	6	14.2				0	N/A		
Ki67 proliferation index			0.371	N/A				< 0.001	0.001
Grade 1	0	N/A				22	53.3		
Grade 2	0	N/A				6	19.0		
Grade 3	6	23.0				4	11		
Grade 4	3	10.0				0	N/A		
Grade 5	15	10.3				6	7.2		

0.001), emergence of extrahepatic metastases at the time of diagnosis (*P* = 0.035), and advanced mitosis grading (*P* = 0.016) were unfavorable prognostic factors for HAS patients. The emergence of extrahepatic metastases at the time of diagnosis (*P* = 0.001), the presence of tumor necrosis (*P* = 0.029), advanced mitosis grading (*P* < 0.001), and a high Ki-67 proliferation index (*P* < 0.001) were unfavorable prognostic factors for HEHE patients. Multivariate analysis showed that the presence of extrahepatic metastases (*P* = 0.043) and histological necrosis (*P* = 0.018) were independent unfavorable prognostic factors for HAS, and the presence of extrahepatic metastases (*P* < 0.001), advanced mitosis grading (P < 0.001), and a high Ki-67 proliferation index (P = 0.001) were independent unfavorable prognostic factors for HEHE.

Discussion

Liver vascular malignancy is normally associated with a history of environmental or occupational exposure to toxicants, but none of our patients had a known history of exposure. Chronic infection with hepatitis B virus, an endemic infectious disease in China commonly considered the etiology of hepatocellular carcinoma and cholangiocarcinoma, was identified

in our patients at a prevalence (approximately 8%) similar to that in the general Chinese population; however, the possibility of hepatitis virus as the etiologic cause of malignant liver vascular tumor has been excluded [8-10]. HAS and HEHE usually manifest as non-specific symptoms, mainly abdominal pain, which are not differentiable from other benign or malignant gastrointestinal disorders. Preoperative laboratory and imaging examinations are also less useful for differentiation of malignant liver vascular tumors [11]. Multiple intrahepatic diseases in HAS patients and especially HEHE patients are often misdiagnosed as metastatic liver tumors or intrahepatic cholangiocarcinoma [12]. Thus, pathological examination is the primary diagnostic modality for HAS and HEHE.

HAS and HEHE exhibit a series of complex histological features similar to those of cavernous and infantile hemangiomas [11, 13, 14]. Pathological diagnosis of HAS and HEHE based on histology of resected specimens will normally give a definitive result; however, epithelioid HAS and HEHE are frequently misdiagnosed as hepatocellular carcinoma or cholangiocarcinoma on needle aspiration biopsy due to the presence of tumor cells enriched with cytoplasm and evident nucleoli [15, 16]. HAS normally destroys the alignment of the hepatocyte plate and forms separate nest-like neoplastic nodules. HAS also has a highly variable cellular atypia and an irregular margin with the normal liver tissue. Endothelial-like HAS cells have a larger cellular body and undergo mitosis more frequently compared to liver sinusoid endothelial cells. A characteristic feature of HAS is that spindle- or oval-shaped tumors grow around the remaining hepatocytes in a scaffold-like structure [1]. In contrast, HEHE has a relatively less destructive effect on the liver tissue, and HEHE cells are interweaved with normal hepatocytes. Epithelioid cells, as a non-specific landmark, can be identified in HEHE as well as HAS in some cases. Dentritic-like cells are often missed in HEHE due to the presence of immature vessels [7]. Moreover, HEHE is more likely to exhibit stromal mucoid, hyaline, or calcified degeneration as well as overdeposition of collagen [7].

Vascular endothelial markers, such as CD31, CD34, and FVIIIRAG, may help to differentiate HAS and HEHE from other non-vascular liver malignancies, although these markers have a limited differential role due to a relatively low sensitivity as shown by our results [3]. The ERG gene is a proto-oncogene located at chromosome 21q22.3 [17, 18] and encoding a long-chain ERG protein. Our results demonstrated a 100% sensitivity of ERG in both HAS and HEHE, with ERG expression mainly located in nuclei for HAS and in nuclei and occasionally the cytoplasm for HEHE, in agreement with the findings in a previous report by Miettinen et al. [4]. The antibody against the N-terminal of ERG protein differs from the counterpart against the C-terminal with respect to specificity [19]. The latter antibody was used in this study and was likely to have cross reactions with cytoplasmic components. To the best of our knowledge, our results demonstrate for the first time that the use of ERG protein in combination with CD31 and CD34 could differentiate liver vascular tumor cells from normal endothelial cells, with liver sinusoid endothelial cells being immunopositive for ERG protein but negative for CD31 and CD34.

ERG protein is a transcription regulator involved in neoangiogenesis and vascular endothelial cell apoptosis through vascular endothelial cadherin (VE-cadherin) [4]. Downregulation of ERG expression inhibits angiogenesis while increasing the number of caspase-positive endothelial cells, and this inhibitory effect can be antagonized by overexpressing VE-cadherin [4]. Our ERG FISH experiment did not identify any known ERG gene rearrangement, such as the ERG-TMPRSS2 gene fusion seen in prostate cancer [7], in HAS or HEHE. Chromosomal translocation t(1;3)(p36.3;q25) was identified in HEHE [20] but remained undetermined in HAS. This finding suggests that HAS and HEHE involve different genetic pathways for malignant angiogenic transformation.

Among multimodal treatments, liver resection is the preferred treatment modality for resectable HAS and HEHE, because it may offer a curative effect and favorable survival as shown by our results. Liver transplantation is also preferred by some investigators due to a long post-transplantation disease-free survival [21, 22]; however, Husted et al. [23] reported a contradictory result that liver transplantation could not improve the overall survival (less than 6 months) of HAS patients, similar to that for 2

HAS patients who underwent liver transplantation in our study. Interventional therapy may be used for palliative treatment of extrahepatic metastases, as this treatment modality has a good local disease control but minimal invasiveness [21]. Generally, single HAS or HEHE without complicating extrahepatic metastases should be surgically treated in combination with liver transplantation and/or interventional therapy if indicated.

HAS and HEHE have a highly varying and generally poor prognosis, and the prognostic factors remain less studied due to the rarity of HAS and HEHE. Locker et al. [24] reported a median survival of 6 months for HAS patients, while long-term postoperative survival of more than 12 months was reported in some cases [25], similar to our results. Compared to HAS, HEHE has a relatively better prognosis as shown by previous reports [9, 26] and our results. Miller et al. [9] reported a 5-year overall survival of 55%, but some patients had an unfavorable prognosis due to a rapidly progressive disease [27]. Early detection and intervention is also crucial for improving the survival of patients with liver vascular malignancies, and a dilemma in surgical practice is that these tumors are unresectable at the time of diagnosis [28]. Our results demonstrate that the presence of extrahepatic metastases led to failure of curative treatment and consequently resulted in poor survival among both HAS patients and HEHE patients. Cardinal et al. [21] and Wang et al. [22] reported that the presence of clinical symptoms and/or extrahepatic metastases is an independent prognostic factor associated with poor survival in HEHE patients. The underlying pathogenesis is that both HAS and HEHE have an occult natural history, and the presence of symptoms and/or metastases signals an advanced disease. Moreover, elevation in carbohydrate antigen 19-9 was reported as an unfavorable prognostic factor for HEHE; this elevation may result from dysregulated bile duct secretion of mucoprotein or secondary cholangitis and indicates a possibility of intra- and extrahepatic metastases [29].

Advanced histological staging is associated with a poor prognosis for HAS and HEHE. Our results showed that patients with an advanced mitosis grading or a high Ki67 proliferation index had a significantly shorter overall survival among both HAS patients and HEHE patients. Meis-Kindblom and Kindblom [30] reported that a Ki67 proliferation index above 10% predicted a poor survival in soft tissue HAS patients. Similarly, a mitotic cell count greater than 2 per 10 HPFs is associated with a poor prognosis in HEHE patients [8, 31]. Our multivariate analysis further demonstrated that mitosis grading and Ki67 proliferation index were independent prognostic factors for HEHE.

There were some limitations in this study. First, the sample size was relatively small due to the rarity of HAS and HEHE; however, to the best of our knowledge, the present work is the largest clinicopathological study of HAS and HEHE to date. Secondly, the specificity of ERG protein remained undetermined in the diagnosis of HAS and HEHE, although this marker had a 100% sensitivity. Therefore, the use of ERG protein in combination with other conventional endothelial markers is expected to improve the diagnostic accuracy for malignant liver vascular tumors. Lastly, it remains to be investigated how the identified prognostic factors can be used to guide the selection of treatment modalities in surgical practice.

In conclusion, HAS and HEHE have an occult and highly varying clinical presentation, and their diagnosis depends on histologic and immuno-histochemical examination. The presence of extrahepatic metastases and advanced mitosis grading are independent unfavorable prognostic factors for HAS and HEHE, and HEHE with a grade 2 mitosis and Ki67 index is associated with a significantly poorer prognosis. ERG protein, as a highly sensitive marker of malignant liver vascular tumors, shows no ERG gene fusion or rearrangement in HAS and HEHE. ERG expression may be a constitutional phenotypic feature unrelated to ERG gene rearrangements in HAS and HEHE, like in other solid organ vascular tumors. However, the possible oncogenic role of ERG in vascular tumors cannot be ruled out, which in combination with other vascular endothelial markers can help to differentiate malignant liver tumors.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Huai-Yin Shi, Department of Pathology, Chinese People's Liberation Army General Hospital, 28 Fuxing Road, Beijing

100853, China. Tel: +86-10-6693-6652-805; Fax: +86-10-6693-6652; E-mail: shihy301@126.com

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