Transcription factor ERG is a specific and sensitive diagnostic marker for hepatic angiosarcoma

Zhan-Bo Wang, Jing Yuan, Wei Chen, Li-Xin Wei

Zhan-Bo Wang, Jing Yuan, Wei Chen, Li-Xin Wei, Department of Pathology, Chinese People’s Liberation Army General Hospital, Beijing 100853, China

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Correspondence to: Li-Xin Wei, MD, Professor, Department of Pathology, Chinese People’s Liberation Army General Hospital, 28 Fuxing Road, Haidian, Beijing 100853, China. weilx301@126.com

Telephone: +86-10-66936652 Fax: +86-10-66939726
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Abstract

AIM: To investigate the expression of ERG, CD34, CD31 (PECAM-1, platelet/endothelial cell adhesion molecule 1) and factor VIII-related antigen (FⅧRAg) in the diagnosis of hepatic angiosarcoma patients.

METHODS: Patient samples were collected from January 1986 to December 2012 from the People’s Liberation Army General Hospital in Beijing, China. We obtained twenty-four samples of hepatic angiosarcoma (HAS) that were confirmed by two pathologist. The samples were the result of three autopsy cases, eight biopsy cases and 13 patients who underwent surgical tumor removal. The HAS cases accounted for 2.23% (24/1075) of all hepatic vascular tumors at the hospital during the same time period. Patient histories including age, gender, clinical manifestations, medical treatments, laboratory tests, radiological images, histological observations and outcomes for each case were analyzed in detail. All samples were evaluated histologically with hematoxylin and eosin staining. Using immunohistochemistry, the expression and localization of ERG was examined in all HAS specimens and compared to the known endothelial markers CD34, CD31 and FⅧRAg. The endothelial markers were also evaluated in a panel of non-HAS tumors.

RESULTS: This cohort of 24 HAS cases is, to the best of our knowledge, currently the largest cohort in the world in the publicly available literature. Hepatic angiosarcoma tissue samples were obtained from 14 males and 10 females with a mean age of 50.6 years (range: 7-86 years). The patients presented with the following clinical manifestations: abdominal pain (16/24), back pain (3/24), heart palpitations (1/20), cough (1/24) or no clinical symptoms (3/24). Tumors were predominantly localized in the right hepatic lobe (15/24) or left hepatic lobe (6/24), or a diffuse growth on the right and left hepatic lobes (3/24). Eleven patients underwent surgical resection (45.8%), two patients received a liver transplant (8.3%), eight patients received interventional therapy (33.3%) and three patients received no treatment (lesions discovered at autopsy, 12.5%). Postoperative follow-up of patients revealed that 87.5% (21/24) of patients had died and three cases were not able to be tracked. In all cases, the mean survival time was 12.1 mo. While 100% of the HAS samples were positive for ERG expression, expression of the other markers was more variable. CD31 was expressed in 79.2% (19/24) of samples, CD34 was expressed in 87.5% (21/24) of samples and FⅧRAg was expressed in 41.7% (10/24) of samples.

CONCLUSION: ERG is a more sensitive and specific diagnostic marker for hepatic angiosarcoma in comparison to CD31, CD34 and FⅧRAg.

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Key words: Liver; Angiosarcoma; ERG; Immunohisto-
Core tip: Hepatic angiosarcoma (HAS) is a rare disease and formal therapeutic guidelines have not been established. A method to detect HAS early using molecular markers would improve patient survival. Here, we have assembled the largest cohort to date of 24 HAS cases to determine if ERG, CD31, CD34 or factor VIII-related antigen (FVIII:RAg) represent a diagnostic marker for the disease. Expression of CD31, CD34 and FVIII:RAg was variable across the 24 samples, while ERG was consistently expressed in all HAS samples. ERG showed increased sensitivity and specificity in comparison to the other markers examined and represents a novel diagnostic marker for HAS.

INTRODUCTION

Angiosarcoma is a malignant neoplasm that affects endothelial cells and can occur in any location throughout the body[1,2]. The location of tumor origin often permits metastases to distant sites. Although hepatic angiosarcoma (HAS) is a very rare disease and accounts for only 2% of primary liver malignancies[3], it is still the third most common primary liver malignancy[4,5]. Case-control studies have shown that approximately one-third of HAS cases appear to be caused by environmental carcinogens via exposure to thorium dioxide, arsenical insecticide or polychlorinated biphenyls[6].

The diagnosis of HAS is difficult, especially if the patient has no history of carcinogen exposure. Long-term survival in HAS patients is poor due to its rapid progression, high recurrence rate and resistance to traditional chemo- and radiotherapies[7-9]. Due to a high recurrence rate and poor surgical outcomes, liver transplantation as a form of therapy for HAS is no longer performed[10]. No formal guidelines exist for the treatment of HAS. Currently, the best treatment option for HAS is partial surgical resection of the liver to remove the tumor. Thus, a means to detect HAS early using specific molecular markers will provide a critical window during which surgical resection can be performed, which will ultimately improve patient survival.

CD31, CD34 and factor VIII-related antigen (FVIII:RAg) are expressed in endothelial cells and have been hypothesized to serve as diagnostic markers of angiosarcoma[10,11]. CD31, a transmembrane glycoprotein adhesion molecule, is expressed by platelets, megakaryocytes, and endothelial cells[12,13]. CD34 is a cell-surface marker expressed in both endothelial cells and hematopoietic stem cells[14]. FVIII:RAg is expressed in Weibel-Palade bodies of endothelial cells and in megakaryocytes[15]. Although these markers are touted as diagnostic for angiosarcoma, the precise role of each of these markers in HAS remains unclear.

ERG (avian v-ets erythroblastosis virus E26 oncogene homolog), a member of the ETS family of transcription factors, is expressed in endothelial cells[16]. ERG plays essential roles in the regulation of angiogenesis and apoptosis of endothelial cells[17,18]. Chromosomal translocations affecting ERG have been implicated in the development of several cancers[19,20]. Recent reports have demonstrated that ERG is a specific and sensitive biological marker in the diagnosis of angiosarcoma[16,20]. The diagnostic value of ERG in HAS is largely unknown and only one previous publication has documented ERG expression in a HAS patient[21]. To this end, we assembled the largest cohort to date of HAS tissues to evaluate the role of ERG expression in HAS. Moreover, we analyzed how ERG expression compares to CD31, CD34 and FVIII:RAg endothelial markers.

MATERIALS AND METHODS

Patients

All patient specimens were collected from January 1986 to December 2012 at the Chinese People’s Liberation Army General Hospital in Beijing, China. We confirmed 24 cases of HAS by pathology after 13 patients underwent surgical tumor removal, eight patients were biopsied and three patients were autopsied. These cases accounted for 2.23% (24/1075) of hepatic vascular tumors during the same time period. Detailed clinical histories, including gender, age, clinical symptoms, tumor location, size, imaging data, and laboratory test results were collected from each patient’s record. Follow-up information on each case was obtained through telephone conversations and hospital records to evaluate patient prognosis in November 2013. As controls, we obtained other types of liver spindle cell tumors, including hepatic epithelioid hemangioendotheliomas (EHE; n = 3), undifferentiated sarcomas (n = 3), leiomyosarcoma (n = 1), sarcomatoid carcinomas (n = 3) and gastrointestinal stromal tumor liver metastases (n = 2).

Histological observations

All hematoxylin and eosin-stained slides were reviewed for each case. Histological changes were evaluated by experienced pathologists using a light microscope.

Immunohistochemistry

Tissues were fixed in 4% formalin and embedded in paraffin for immunohistochemistry using the EnVision kit (DAKO; Denmark). The antibodies used in this study are listed in Table 1.

Statistical analysis

Statistical analysis was performed using the SPSS 17.0 statistical software package (SPSS Inc., Chicago, IL, United States). Cumulative survival curves were generated us-
Table 1 Antibodies used for expression analysis in hepatic angiosarcoma samples

| Protein name | Antibody clone | Source | Concentration | Location |
|--------------|----------------|--------|---------------|---------|
| Vimentin     | V9             | Invitrogen | 1:200       | C       |
| CD31         | J-70A          | Gene Tech | 1:100        | M       |
| CD34         | QBEnd-10       | Dako    | 1:50          | M       |
| FVIIIIRAg    | F8/86          | Dako    | 1:50          | C       |
| ERG          | Ep111          | Epitomics | 1:100        | N       |
| CK           | Polyclonal     | Dako    | 1:100         | C       |
| GPC-3        | IG12           | Cell marque | 1:150      | C,M     |
| Hepatocyte   | OCH115         | Zeta    | 1:100         | C       |
| CD117        | Polyclonal     | Dako    | 1:400         | M       |
| Desmin       | D33            | Dako    | 1:100         | C       |
| Ki-67        | MB-1           | Dako    | 1:200         | N       |

C: Cytoplasm; M: Membrane; N: Nucleus; FVIIIIRAg: Factor Ⅷ-related antigen; CK: Pan-cytokeratin; GPC-3: Glypican-3; CD117: C-kit proto-oncogene.

The Kaplan-Meier method. The hazard ratio and 95% confidence interval were also calculated.

RESULTS

Clinical data of the HAS cohort

The HAS tissues were obtained from 14 males and 10 females, with a mean age of 50.6 (range: 7-86) years (Table 2). The patients presented with the following clinical manifestations: abdominal pain (16/24), back pain (3/24), heart palpitations (1/20), cough (1/24) or no clinical symptoms (3/24). Tumors were located in the right lobe (15/24), left hepatic lobe (6/24) or a diffuse growth on the right and left hepatic lobes (3/24) (Table 2). Patients underwent surgical resection (11/24), liver transplantation (2/24), interventional therapy (8/24) or they received no treatment (lesions discovered at autopsy; 3/24). Postoperative follow-up of each case showed that 18 patients had died and three cases could not be accounted for. Kaplan-Meier survival curves demonstrated that the prognosis of HAS cases was poor (Figure 1, Table 3). Laboratory tests for alpha-fetoprotein, carcinoembryonic antigen, CA19-9, and CA125 cases was poor (Figure 1, Table 3). Laboratory tests for alpha-fetoprotein, carcinoembryonic antigen, CA19-9, and CA125, respectively (Figure 4B-D). Moreover, only one case was positive for pan-cytokeratin (CK) expression (1/24). The cell proliferation index was assayed by Ki-67 staining. In all samples, the cell proliferation index was greater than 10% and up to 60%. Desmin, c-kit proto-oncogene (CD117) and Glypican-3 (GPC-3) expression was absent in all samples.

Expression of ERG, CD31, CD34 and FVIIIIRAg in hepatic angiosarcoma tumors

To evaluate ERG, CD31, CD34 and FVIIIIRAg as diagnostic markers of HAS, we evaluated their expression by immunohistochemistry. ERG expression was observed in all HAS samples examined (24/24; Figure 4A). In contrast, the other three endothelial markers examined were not uniformly expressed across HAS samples. CD34, CD31, and FVIIIIRAg were expressed in 21/24, 19/24 and 10/24 cases, respectively (Figure 4B-D). Moreover, only one case was positive for pan-cytokeratin (CK) expression (1/24). The cell proliferation index was evaluated using the Kaplan-Meier method. The hazard ratio and 95% confidence interval were also calculated.

Tumor histology

When tumor histology was examined, one layer or multiple layers of tumor cells lined a vascular lumen and gave rise to the lumen-like structure (Figure 3A and B). The tumor cells grew along the sinusoids, with the liver serving as a scaffold for tumor growth (Figure 3C). Three of the samples had overt compartments that were filled with red blood cells. The tumors predominantly contained atypical spindle cells and mitotic cells were frequently observed. Moreover, we observed significant necrosis and calcification within the tumors. Areas of epithelial differentiation were apparent in some tumors. In patients with recurrent tumors following liver transplantation, the tumors contained a large blood chamber that differed from the morphology observed in the primary lesion.
absent. Both gastrointestinal stromal tumor liver metastases were positive for CD34, but lacked ERG, CD31 and FⅧRAg.

Table 2  Clinical and pathological features of the hepatic angiosarcoma cohort

| No. | Gender | Age/yr | Clinical symptoms | Tumor location | Maximum tumor size/cm | Treatment methods | Follow-up |
|-----|--------|--------|-------------------|----------------|------------------------|------------------|-----------|
| 1   | Male   | 42     | Abdominal discomfort | Left lateral lobe of the liver | 5 × 3 × 3 | Adjuvant therapy | Spleen metastasis at treatment and died 10 mo later |
| 2   | Female | 30     | Abdominal discomfort | Left inner hepatic lobe | 6 × 4 × 3 | Adjuvant therapy | Unable to follow-up after 9 mo |
| 3   | Male   | 60     | Upper abdominal pain | Right hepatic lobe | 16 × 15 × 13 | Surgery | Died after 12 mo |
| 4   | Female | 58     | Liver pain | Left lobe of liver | 9.5 × 9.4 × 6 | Adjuvant therapy | Unable to follow-up after 6 mo |
| 5   | Female | 52     | Back pain | Left and right hepatic lobes | 13 × 8 × 6 | Adjuvant therapy | Died 8 mo later |
| 6   | Male   | 7      | Abdominal pain, fever | Right hepatic lobe | 7 × 6 × 3 | Surgery | Died 1 mo later |
| 7   | Female | 52     | Upper abdominal pain | Left hepatic lobe | 12.5 × 12 × 4 | Surgery | Post-operative thoracic and lung metastases and died 31 mo later |
| 8   | Female | 62     | Abdominal discomfort | Left hepatic lobe | 7.5 × 6 × 3 | Surgery | Post-operative lung metastases and died 34 mo later |
| 9   | Male   | 68     | Upper abdominal pain | Right hepatic lobe | 10 × 8 × 6 | Adjuvant therapy | Portal vein cancer thrombosis at diagnosis and died 9 mo later |
| 10  | Female | 29     | Hypo-proteinemia | Left and right hepatic lobes | 27 × 20 × 9 | Liver transplant | Spleen metastasis at diagnosis and died during surgery |
| 11  | Male   | 49     | Asymptomatic | Right hepatic lobe | 3 × 3 × 2 | Surgery | Bone metastases and died 43 mo later |
| 12  | Female | 62     | Upper abdominal pain | Right hepatic lobe | 3 × 2 × 2 | Liver Transplant | Recurrence of tumor in the transplanted liver and died 4 mo later |
| 13  | Female | 32     | Asymptomatic | Right hepatic lobe | 6 × 5 × 3 | Surgery | Died 3 mo later |
| 14  | Female | 51     | Upper abdominal pain | Right hepatic lobe | 10 × 6 × 5 | Surgery | Died 8 mo later |
| 15  | Male   | 53     | Upper abdominal pain | Left hepatic lobe | 27 × 17 × 6 | Surgery | Recurrence after 4 mo and died 7 mo later |
| 16  | Male   | 49     | Upper abdominal pain | Right hepatic lobe | 11 × 7 × 5 | Surgery | Died 10 mo later |
| 17  | Female | 66     | Back pain | Right hepatic lobe | 6 × 5 × 4 | Adjuvant therapy | Thoracic metastasis at diagnosis and died 5 mo later |
| 18  | Male   | 86     | Upper abdominal pain | Left and right hepatic lobes | 8 × 7 × 3 | Autopsy findings without treatment | Died due to tumor rupture |
| 19  | Male   | 86     | Upper abdominal pain | Right hepatic lobe | 9 × 9 × 6 | Autopsy findings without treatment | Tumor metastasis and died due to heart failure |
| 20  | Male   | 70     | Palpitations | Right hepatic lobe | 4.5 × 4 × 3 | Autopsy findings without treatment | Tumor metastasis and died due to heart failure |
| 21  | Female | 45     | Cough | Right hepatic lobe | 4.5 × 4 × 4 | Adjuvant therapy | Lung metastasis at diagnosis and died 6 mo later |
| 22  | Male   | 21     | Right shoulder pain | Right hepatic lobe | 16.5 × 15 × 8 | Surgery | Tumor recurrence and died 8 mo later |
| 23  | Male   | 21     | Upper abdominal pain | Right hepatic lobe | 3.5 × 3.5 × 3 | Adjuvant therapy | Unable to follow-up after 10 mo |
| 24  | Male   | 63     | Upper abdominal pain | Right hepatic lobe | 3.5 × 3.5 × 3 | Surgery | Tumor recurrence after 18 mo and died 21 mo later |

1Patients who received percutaneous biopsies.

Figure 2  Magnetic resonance imaging and whole-mount images of hepatic angiosarcoma. A: Magnetic resonance imaging of a hepatic angiosarcoma (HAS) patient showed a significantly increased volume in the left lobe of the liver and an abnormal signal shadow with heterogeneous enhancement; B: The HAS tumor showed honeycomb morphology with an ill-defined border.
Figure 3  Analysis of hepatic angiosarcoma histology with hematoxylin and eosin staining. A: The tumor was similar in appearance to a cavernous hemangioma on the inside and was lined by endothelial cells of similar shapes (× 200); B: Endothelial cells are distended with a spindle or polygonal morphology. Frequent mitotic events (indicated by arrow) and a pale eosinophilic cytoplasm were also observed (× 300); C: Tumor cells were observed growing around the vascular lumen and formed a vascular lumen-like structure (× 300).

Figure 4  Expression of transcription factor ERG, CD31 (platelet/endothelial cell adhesion molecule 1), CD34 and factor VIII-related antigen in hepatic angiosarcoma tissues. A: Positive expression was observed for nuclear ERG expression in hepatic angiosarcoma (HAS) (× 300); B: Membranous CD34 staining in HAS (× 300); C: Membranous CD31 staining in HAS; D: Cytoplasmic factor VIII-related antigen in hepatic angiosarcoma staining in HAS (× 300).
Table 3  Means for survival time

| Factor | Mean1 Estimate | Std. error | 95% CI | Lower bound | Upper bound |
|--------|----------------|-------------|--------|-------------|-------------|
| 1      | 12.196         | 2.788       |        | 6.731       | 17.668      |

1Estimation was limited to the largest survival time if it is censored.

**DISCUSSION**

Primary sarcomas of the liver are rare. The most common hepatic sarcomas are HAS, embryonal sarcoma, leiomyosarcoma, EHE, fibrosarcoma and malignant fibrous histiocytoma. HAS is primarily observed in the elderly and often presents with nonspecific symptoms such as discomfort or distension of the abdomen, weight loss or fatigue, which makes the diagnosis difficult. HAS is frequently caused by exposure to carcinogens such as thorium dioxide, arsenical insecticide or polyvinyl chloride. However, in all the Chinese patients that comprised our cohort, carcinogen exposure was not an important factor in disease onset. Our data are consistent with reports on HAS development from Taiwan and South Korea. To better address the role of carcinogens and HAS in China, an approach involving a survey of multiple medical centers across the country would be beneficial. Infection from hepatitis B virus (HBV) has also been implicated as a risk factor for HAS. In our study, the frequency of HBV infection was no different to that in the normal, healthy Chinese population, indicating that HBV infection did not play a role in HAS onset.

HAS often appears as a single mass when viewed histologically, however, multiple masses have also been observed and HAS may affect the whole liver. In the clinic, four primary patterns of disease have been observed: (1) multiple nodules; (2) a large dominant mass; (3) a dominant mass combined with multiple nodules; and (4) diffuse micronodular infiltration of the liver, although this is observed less frequently. In the HAS patient cohort, a large dominant mass was most frequently observed (62.5%). The remaining patients had a dominant mass and multiple nodules (18.8%), multiple nodules (12.5%) or diffuse micronodular infiltration of the liver (6.3%). When HAS samples are viewed by microscopy, the tumor cells are often mitotic, have a spindle or polygonal morphology and may be in a single layer or multi-layered surrounding a vascular source, connected with thick hepatic cables. HAS tumor cells have the ability to form papillary structures and may show epithelial differentiation.

HAS patient survival is currently very poor, with a median survival of 6 mo without treatment. Even with treatment, only 3% of patients are reported to survive longer than 2 years. Poor prognosis of HAS patients is primarily due to early metastases and an extended time between disease onset and correct diagnosis. Surgical resection is currently the standard treatment for HAS. Early diagnosis of HAS is required for surgical resection to be beneficial to the patient. Other treatments such as chemotherapy or radiotherapy have not shown a conclusive survival benefit in HAS patients. Thus, the identification of specific and sensitive biomarkers to diagnose HAS would significantly improve patient outcome. Here, we have assembled the largest HAS cohort to date and provide evidence that ERG is a specific diagnostic marker for HAS.

ERG regulates angiogenesis and differentiation of embryonic stem cells into endothelial cells. Chromosomal translocations affecting ERG expression have been shown to play a role in the development of several cancers, including prostate cancer. The diagnostic value of ERG in HAS, however, has not been evaluated. Here, we have shown that ERG expression was detected in all HAS cases examined. While three cases of EHE, one of our controls, also showed positive expression of ERG, the remaining controls-undifferentiated sarcoma, sarcomatoid carcinoma and gastrointestinal stromal tumor liver metastases-were negative for ERG expression. These results suggest that ERG expression is relatively specific to HAS tumors. In comparison to the other endothelial markers (i.e., CD31, CD34 and FVIII RAg) examined, ERG was more sensitive and had increased specificity for HAS. Thus, ERG may represent a novel diagnostic marker for HAS. Because ERG expression shows a similar pattern in both benign and malignant vascular populations, histological examination is required to differentiate angiosarcomas from other endothelial neoplasms. Tumor cell mitotic activities, atypical mitotic features and necrosis have been fundamentally recognized as probable factors determining propensity for HAS.

In conclusion, ERG is a new biomarker for the diagnosis of HAS. While CD31, CD34 and FVIII RAg were variably expressed across our HAS cohort, ERG was expressed in all tumors examined. Because HAS is a relatively rare malignancy, our cohort (n = 24) is, to the best of our knowledge, the largest in the world to date. The data provided from this patient cohort will help to establish a standard for symptoms, diagnosis and treatment of HAS. Moreover, our data implicate ERG as a potent marker in the diagnosis of HAS tumors.

**COMMENTS**

**Background**

Hepatic angiosarcoma (HAS) is a rare disease characterized by poor prognosis due to its rapid progression, high recurrence rate and resistance to traditional chemotherapies. Surgical resection, which requires a prompt diagnosis, is currently the best treatment option for HAS. Because the initial symptoms of HAS can be nonspecific, it is difficult to diagnose. Thus, a specific molecular marker for the early diagnosis of HAS would provide a critical time window for surgical resection and greatly improve patient survival. ERG is a novel biomarker in HAS that has been implicated in other cancers, but its value as a diagnostic marker in HAS has not yet been evaluated.
Research frontiers
ERG is a member of the ETS family of transcription factors and is expressed in endothelial cells. Angiogenesis, endothelial cell differentiation and endothelial homeostasis are regulated by ERG. Previous reports have shown that ERG is a specific and sensitive biological marker for the diagnosis of angiosarcoma. The diagnostic value of ERG expression in HAS has not been evaluated. In addition, because HAS is quite rare, it has been difficult to evaluate diagnostic criteria in a large patient cohort.

Innovations and breakthroughs
This study has assembled the largest HAS cohort to date (n = 24 cases) to evaluate the role of ERG expression in liver angiosarcoma. Moreover, this study compares ERG expression to other known endothelial markers such as CD31, CD34 and factor VIII-related antigen (FVIII-RAg) as a means of HAS diagnosis. While CD31, CD34 and FVIII-RAg have variable expression across the cohort samples, ERG expression was found in 100% of the cases examined. The data provided from this patient cohort will help to establish a standard for symptoms, diagnosis and treatment of HAS. This is the first study to identify ERG expression as a marker for HAS.

Applications
The findings in this study highlight ERG expression as a potent and novel marker for HAS tumors, which will aid in developing diagnostic tests for the disease.

Terminology
HAS is a rare type of cancer that affects the endothelial cells of the liver and is difficult to diagnose until the disease has spread. ERG, CD31, CD34 and FVIII-RAg are genes expressed in endothelial cells, the cell population affected in HAS, and may therefore represent a way of identifying HAS tumors.

Peer review
This is a good original research article that demonstrates that ERG is a specific and sensitive diagnostic biomarker for hepatic angiosarcoma. Moreover, ERG is a more sensitive and specific diagnostic biomarker for hepatic angiosarcoma in comparison to CD31, CD34 and FVIII-RAg. This study represents the largest cohort of HAS cases to date and is well designed to evaluate the value of ERG in HAS diagnosis. The results of this study have a high potential for clinical application.

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