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Pediatric hepatic hemangiosarcoma in a rhesus macaque (*Macaca mulatta*)

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Abstract

Background—Pediatric hepatic angiosarcoma is a rare condition in children with poor prognosis. Microscopically this neoplasm has a particular ‘Kaposi-form’ arrangement. Hemangiosarcoma in non-human primates is a rare finding.

Methods—Gross and microscopic examination of a 3-year-old rhesus were performed. Immunohistochemistry was used to characterize the hepatic hemangiosarcoma.

Results—The gross necropsy revealed hemoabdomen and a 4 × 3 × 3 cm mass in the liver with multiple smaller masses throughout the hepatic parenchyma. Histopathology confirmed a poorly differentiated hemangiosarcoma. Other organs submitted were free of metastases.

Conclusions—Hemangiosarcoma in non-human primates has been rarely reported. Diagnosis was confirmed by expression of endothelial-specific markers CD31 and vWF by immunohistochemistry. Due to the young age of this monkey and the particular solid pattern throughout the mass this neoplasm resembles pediatric hepatic angiosarcoma in humans.

Keywords

hemangiosarcoma; immunohistochemistry; liver neoplasms; *Macaca mulatta*; pediatric hepatic angiosarcoma

Introduction

Hemangiosarcoma (HSA) is a malignant, metastatic vascular tumor composed of pleomorphic neoplastic endothelial cells forming vascular spaces [1,2]. HSA is the most common splenic neoplasm in dogs with fewer reports in cats, horses, ferrets, birds, and rarely in non-human primates [2-8]. Similar types of neoplasms occur in people referred to as hemangiomas, hemangioendotheliomas, and angiosarcomas [3,6,7]. The pediatric presentation of hepatic angiosarcoma is particularly uncommon with just 41 reports in the literature, and poor prognosis with just three surviving patients after oncology treatment [4]. We report a case of hepatic hemangiosarcoma in a juvenile rhesus macaque found at necropsy following spontaneous death. The histological pattern of the hepatic hemangiosarcoma and the age of the animal resemble the human pediatric angiosarcoma.

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Materials and methods

A 3-year-old female rhesus macaque with unremarkable clinical history and group housed in specific pathogen-free (SPF) breeding colony was found dead in its corral, and a necropsy was performed. The animal was born and housed at the Caribbean Primate Research Center (CPRC, Puerto Rico). SPF animals at CPRC are screened and negative by serology for simian herpes B virus, measles, simian immunodeficiency virus, simian T-cell leukemia virus-1, simian retrovirus group D and simian virus-40. The CPRC clinical veterinarian performed the postmortem examination and tissue samples including liver, spleen, and heart were submitted to the New England Primate Research Center for histopathological evaluation.

Tissues were fixed in 10% phosphate-buffered formalin for histological and immunohistochemical analysis. Tissue was paraffin embedded, and sectioned at 5 μ m for hematoxylin and eosin and immunohistochemistry staining. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue sections of the hepatic neoplasm using an avidin–biotin complex method (ABC Standard; Vector Laboratories, Burlingame, CA) with diaminobenzadine (DAB; Dako Corporation, Carpinteria, CA) as the chromagen. Antibodies to endothelial markers, platelet/endothelial cell adhesion molecule 1 (PECAM-1/CD-31, IgG1; LabVision, Fremont, CA) and von Willebrand's factor (fVIII/vWF, IgG1; Dako), were used at dilutions 1:100 and 1:50, respectively, overnight at 4°C after proteinase K pretreatment. Cellular proliferation was assessed with anti-MIB-1 (Ki-67, IgG1; Dako) with microwave antigen retrieval and incubation at 1:80 dilution overnight at 4°C. Alteration in expression of the tumor suppressor protein p53 (IgG2b; Dako) was examined with microwave antigen retrieval and incubation at 1:390 dilution overnight at 4°C. To assess the possible etiologic association with rhesus rhadinovirus (RRV) or retroperitoneal fibromatosis (RF)-associated herpesvirus (two rhesus γ herpesviral homologues to HHV8), tissues were incubated with anti-latent nuclear antigen 1 from human herpesvirus-8 (HHV-8)/Kaposi sarcoma herpes virus (LANA-1; Advanced Biotechnologies, Columbia, MD) at 1:850 dilution overnight at 4°C. Step sections were incubated with isotype-matched irrelevant antibodies as negative controls. Signals were detected with the chromagen DAB and slides were counterstained with Mayer's hematoxylin.

Results

Gross findings included significant hemoabdomen and several variably sized (5 mm to 4 cm diameter) dark brown masses in the liver with multiple blood-filled cavitations (Fig. 1A,B). One of the masses on the surface of the liver ruptured resulting in hemoabdomen. All other organs were grossly normal.

Histologically, the liver had numerous, round, variably sized, individual to coalescing foci of an invasive neoplasm that compressed and replaced the hepatic parenchyma. Collapsed stromal fibrous connective tissue partially encapsulated some of the neoplastic foci. The neoplasm was highly cellular with markedly pleomorphic spindle to round cells (endothelial) forming variably sized, blood-filled vascular channels (Fig. 1C,D). In addition, there were multiple densely cellular areas with neoplastic sarcomatous cells forming whorls of 'kaposi-form' spindle cells consistent with human pediatric hepatic angiosarcoma (Fig. 1E) [4]. Individual neoplastic cells contained moderate to abundant pale eosinophilic cytoplasm with distinct borders, plump to elongate nuclei with coarsely clumped chromatin, and one to two nucleoli. Mitotic figures were rare. There were multiple areas of necrosis, hemorrhage, and variable amounts of fibrin within the neoplastic foci. Diffusely in the hepatic parenchyma and adjoining the neoplastic foci the sinusoids were dilated and the portal areas were surrounded by moderately thick fibrous connective tissue admixed with lymphocytes and neutrophils. The hepatic capsule was multifocally thickened by fibrous connective tissue and multiple neoplastic

vascular formations. Based on the macroscopic and microscopic findings, a diagnosis of poorly differentiated HSA was made. Other changes, significant microscopic findings included scattered erythropoietic cells throughout the hepatic parenchyma and moderate lymphoid depletion and extramedullary hematopoiesis in the spleen.

Immunohistochemistry confirmed the endothelial origin of the hepatic neoplasm with strong immunoreactivity in 90% of the cells to CD31 (PECAM, platelet endothelial cell adhesion molecule) (Fig. 1F) and variable immunoreactivity of 20% of the cells to vWF (Fig. 1G). Strong immunopositivity to p53 in 65–75% of neoplastic cells (Fig. 1H) implicates involvement of aberrant nuclear expression of the tumor suppressor protein in tumor pathogenesis. Neoplastic cells also exhibited increased proliferation with strong immunoreactivity to MIB-1 (Ki-67) in 30–40% of the cells (Fig. 1I). No association was determined with a γ herpesvirus as LANA-1 was negative (data not shown).

Discussion

This case of hepatic HSA in a non-human primate represents an extremely uncommon finding. Tumors of the cardiovascular system in non-human primates are exceptionally rare with previous reports including a rhesus macaque with subcutaneous HSA and a renal HSA in a mustached tamarin, a few reports of hemangiomas in rhesus at different locations, and a cavernous lymphangioma in a squirrel monkey [2,5,6,8]. There is a report of hemangioendothelioma from a superficially irradiated rhesus and a hepatic hemangioendothelial sarcoma in a cynomolgus macaque exposed to aflatoxin B1 [8,9].

In humans, angiosarcoma is the most common term used to define both hemangiosarcoma and lymphangiosarcoma. Human hepatic angiosarcoma (HAS) is a rare neoplasia with an incidence of 0.1 to 0.25 per million in the general population with increasing prevalence associated with some environmental and occupational pollutants [10]. Exposure to Thorotrast® Testagar & Co., Inc, Detroit, Michigan (thorodium dioxide, a radio-active element used as a radiologic contrast media), vinyl chloride monomer (a substrate used in the plastic industry), and arsenic are the most commonly reported substances associated with HAS, along with other substances including steroids, diethylstilbestrol, phenelzine, urethane, cyclophosphamide, inorganic copper and oral contraceptives [10,11]. Other risk factors include radiation therapy, hormonal status (in breast angiosarcoma), foreign materials (e.g., bone wax, Dacron, metal bodies, surgical sponges), lymphedema (post-surgical, e.g., Stewart–Traeves syndrome, Kettles syndrome), and chronic lymphangiedema (genetic or idiopathic).

The presentation of human HAS is typically multiple foci in the liver with metastases to the spleen, lung, bone, lymph nodes, and adrenals [10]. Angiosarcoma in humans is confirmed by immunohistochemistry by vWF, CD31/PECAM-1, CD34, and by electron microscopy [12, 13].

Other immunohistochemical markers have also been used to characterize angiosarcomas and their prognosis. MIB-1 (Ki-67), a nuclear protein present in all non-G0 phases of the cell cycle, has been used to assess the prognosis of a variety of tumor lesions, including breast hemangiomas and hemangiosarcomas [13]. Aberrant expression of the tumor suppressor genes p53 and retinoblastoma (Rb) also have been associated with hemangiosarcoma and other human cancers [11,14]. While an etiology is not known for this class of tumors, there is a report of HHV8 association with angiosarcoma [15]; however, a more extensive study of 40 cases of human angiosarcoma failed to demonstrate such as association [16].

A subtype of human angiosarcomas include pediatric hepatic vascular tumors comprising infantile hemangioendothelioma, cavernous hemangioma, epithelioid hemangioendothelioma, and angiosarcoma [4]. Infantile hemangioendothelioma is the third most common pediatric

hepatic tumor, accounting for 12% of all liver neoplasias, while the other types are very rare [4]. Pediatric hepatic angiosarcomas are characterized histopathologically by hypercellular whorls of 'kaposi-form' sarcomatous spindloid cells (Fig. 1,E), in addition to the vascular formation of the adult counterpart. These tumors have a poor prognosis with average survival of 16 months, affecting twice as many females as males, with no clear association with environmental pollutants [4].

The morphological and histopathological features of the hepatic tumor of this 3-year-old rhesus closely resemble those of pediatric hepatic angiosarcoma in children. The age of the animal and the lack of exposure to risk factors (pollutants, radiation, etc.) associated to the morphological features of the pediatric primate hepatic tumor described in this report make a close parallel with the presentation in children.

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References

1. Jones, TC.; Hunt, RD.; King, NW. Veterinary Pathology. Baltimore, MD: Williams & Wilkins; 1997.
2. Myers DD Jr, Dysko RC, Chrisp CE, Decoster JL. Subcutaneous hemangiosarcomas in a rhesus macaque (*Macaca mulatta*). J Med Primatol 2001;30:127–30. [PubMed: 11491406]
3. Darby C, Ntavlourou V. Hepatic hemangiosarcoma in two ferrets (*Mustela putorius furo*). Vet Clin North Am Exot Anim Pract 2006;9:689–94. [PubMed: 16931391]
4. Dimashkieh HH, Mo JQ, Wyatt-Ashmead J, Collins MH. Pediatric hepatic angiosarcoma: case report and review of the literature. Pediatr Dev Pathol 2004;7:527–32. [PubMed: 15547777]
5. Gozalo A, Chavera A, Dagle GE, Montoya E, Weller RE. Primary renal hemangiosarcoma in a moustached tamarin. J Med Primatol 1993;22:431–2. [PubMed: 8169946]
6. King CS, Streett JW, Brownstein DG. Cavernous lymphangioma in a squirrel monkey. Lab Anim Sci 1993;43:252–4. [PubMed: 8355487]
7. North PE, Waner M, Buckmiller L, James CA, Mihm MC Jr. Vascular tumors of infancy and childhood: beyond capillary hemangioma. Cardiovasc Pathol 2006;15:303–17. [PubMed: 17113009]
8. Woodruff JM, Johnson DK. Hepatic hemangioendothelioma in a rhesus monkey. Pathol Vet 1968;5:327–32. [PubMed: 5750002]
9. Fujimoto Y, Hampton LL, Luo LD, Wirth PJ, Thorgeirsson SS. Low frequency of p53 gene mutation in tumors induced by aflatoxin B1 in nonhuman primates. Cancer Res 1992;52:1044–6. [PubMed: 1310637]
10. van Kampen RJ, Erdkamp FL, Peters FP. Thorium dioxide-related haemangiosarcoma of the liver. Neth J Med 2007;65:279–82. [PubMed: 17890786]
11. Weihrauch M, Markwarth A, Lehnert G, Wittekind C, Wrbitzky R, Tannapfel A. Abnormalities of the ARF-p53 pathway in primary angiosarcomas of the liver. Hum Pathol 2002;33:884–92. [PubMed: 12378512]
12. Burrows PE, Dubois J, Kassarian A. Pediatric hepatic vascular anomalies. Pediatr Radiol 2001;31:533–45. [PubMed: 11550763]
13. Shin SJ, Lesser M, Rosen PP. Hemangiomas and angiosarcomas of the breast: diagnostic utility of cell cycle markers with emphasis on Ki-67. Arch Pathol Lab Med 2007;131:538–44. [PubMed: 17425381]
14. Yonemaru K, Sakai H, Murakami M, Yanai T, Masegi T. Expression of vascular endothelial growth factor, basic fibroblast growth factor, and their receptors (flt-1, flk-1, and flg-1) in canine vascular tumors. Vet Pathol 2006;43:971–80. [PubMed: 17099154]

15. Gessi M, Cattani P, Maggiano N, Scambia G, Lauriola L, Ranelletti FO, Ricci R. Demonstration of human herpesvirus 8 in a case of primary vaginal epithelioid angiosarcoma by in situ hybridization, electron microscopy, and polymerase chain reaction. *Diagn Mol Pathol* 2002;11:146–51. [PubMed: 12218453]
16. Schmid H, Zietz C. Human herpesvirus 8 and angiosarcoma: analysis of 40 cases and review of the literature. *Pathology* 2005;37:284–7. [PubMed: 16194826]

Fig. 1.

(A, B) Liver; rhesus macaque. Multifocal 5 mm to 4 cm in diameter blood-filled neoplastic masses compressing adjacent normal hepatic parenchyma. (C) Liver; rhesus macaque. Hepatic hemangiosarcoma: coalescing foci of an invasive neoplasm that compresses and replaces the hepatic parenchyma with blood-filled spaces lined by neoplastic endothelial cells. Hematoxylin and eosin. (D) Liver; rhesus macaque. Hepatic hemangiosarcoma: markedly pleomorphic spindle to round endothelial cells forming variable sized, blood-filled vascular channels. Hematoxylin and eosin. (E) Liver; rhesus macaque. Hepatic hemangiosarcoma: densely cellular areas with neoplastic sarcomatous cells forming whorls of 'kaposi-form' spindle cells consistent with PHAS. Hematoxylin and eosin. (F) Liver; rhesus macaque. Hepatic hemangiosarcoma: strong cytoplasmic reactivity for CD31 in the neoplastic epithelial cells surrounding entrapped normal hepatocytes and portal areas. DAB with Mayer's hematoxylin counterstain. (G) Liver; rhesus macaque. Hepatic hemangiosarcoma: endothelial neoplastic cells exhibit weak immunoreactivity for vWF antigen. DAB with Mayer's hematoxylin counterstain. (H) Liver; rhesus macaque. Hepatic hemangiosarcoma: neoplastic endothelial cells express increased aberrant nuclear p53 protein. DAB with Mayer's hematoxylin counterstain. (I) Liver; rhesus macaque. Hepatic hemangiosarcoma: endothelial cells express the proliferation marker MIB-1/Ki-67. DAB with Mayer's hematoxylin.