

## Case Report

# Subcutaneous hemangiosarcomas in a rhesus macaque (*Macaca mulatta*)

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**Abstract:** Hemangiosarcoma is a malignant tumor of vascular endothelial cell origin. The occurrence of hemangiosarcoma in nonhuman primates has been rarely documented. An adult male rhesus monkey was reported having a firm subcutaneous swelling, approximately 4.5 cm in diameter, on the ventral midline of the abdomen. Fine-needle aspiration, microbial culture, biopsy, radiographs, exploratory laparotomy, histopathology, immunohistochemistry, hematology, and serology were performed. A second subcutaneous mass approximately 4.5 × 4.0 × 2.7 cm developed on the ventral midline several weeks later. A fine-needle aspirate of the first mass consisted of numerous erythrocytes with few polymorphonuclear cells and lymphocytes. Histopathology showed foci of spindle-shaped cells surrounding the vascular spaces. Many spindle-shaped cells had prominent nucleoli, and mitotic figures could occasionally be seen. Immunohistochemical staining of the masses for Factor VIII-related antigen, an endothelial cell and tumor marker, yielded positive results. Both masses were consistent with hemangiosarcoma.

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## Introduction

Hemangiosarcoma is a malignant tumor of vascular origin, formed by proliferation of endothelial tissue lining irregular vascular channels [3]. Cells of this tumor type range from small to medium size and/or spindle to stellate shape. Diagnostic cytological evaluation of cells from hemangiosarcomas varies in morphology from normal endothelial cells to cells with marked variation in size, nuclear and nucleolar size, increased nucleus:cytoplasm ratio, nucleolar prominence, nucleolar angularity, and cytoplasmic basophilia [2, 5]. Aspirates from human patients evaluated in a recent study were characterized by hypocellularity, round or spindle-shaped cells, and round to spindle-shaped nuclei in a bloody background [8]. Hemangiosarcomas have a predilection for parenchymatous organs and cutaneous tissue with metastases commonly involving the mesentery and lungs. Cases have been reported in humans, horses, dogs, and cats [1, 5, 6, 10]. The occurrence of hemangiosarcomas in nonhuman primates has rarely been reported [15]. Previously documented cases include a renal hemangiosar-

coma in a moustached tamarin (*Saguinus mystax*) [4], and a cavernous hemangioma affecting the ovary in a rhesus monkey [9]. The specific purpose of this report is to document the first case of recurrent subcutaneous hemangiosarcoma in a rhesus monkey.

## Materials and methods

An 8-year-old male rhesus macaque (*Macaca mulatta*), 11.5 kg in weight, was reported to the veterinary staff for having a firm swelling on the ventral midline of the abdomen cranial to the umbilicus (Fig. 1). The animal was housed in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, National Academy Press, Washington, DC, 1996), in an AAALAC-accredited animal facility at the University of Michigan. This animal was housed individually in a stainless steel nonhuman primate cage and was provided a commercial primate diet (Lab Diet® 5038; PMI® Nutrition International, Inc., Brentwood, MO) and water via lixit *ad libitum*.

This rhesus monkey was part of an ongoing drug discrimination protocol and had a previous history of long-term venous catheterization with some resultant infections. This nonhuman primate was in sound condition and good health based on physical examination. The firm swelling on the ventral midline was caused by a subcutaneous mass, approximately  $4.5 \times 5.0 \times 1.75$  cm. A piece of cellophane was placed over the mass and a perimeter tracing was made to record any change of mass size over time. Two weeks later the mass had increased in size to approximately  $6.5 \times 4.5 \times 2.5$  cm. A fine-needle aspirate of the mass yielded 0.75 ml of turbid red fluid with black particulate. This sample was submitted for cytology and microbial culture. Survey radiographs were taken to evaluate the primate's thorax and abdomen for abnormalities. For surgical resection of the mass, the monkey was immobilized with 10 mg/kg of ketamine HCl (100 mg/ml; Fort Dodge Animal Health, Fort Dodge, IA) by intramuscular injection before inhalation anesthesia with isoflurane (Aerrane, Fort Dodge Animal Health, Fort Dodge, IA) at 1.5–2.0% mixed with 100% oxygen. A wide circumferential resection was performed so that a 3.0-cm margin of normal tissue was excised with the tumor and surrounding fascia [6, 7, 11]. At this time an exploratory laparotomy was also performed to grossly evaluate abdominal organs for tumors. A layered closure of simple interrupted and subcuticular sutures was used to close the skin defect and eliminate dead space [12]. The mass was then fixed in 10% buffered formalin and submitted for histopathologic evaluation. A serum sample was taken from the primate and submitted for a determination of antibody titers to simian retroviruses: SIV, STLV-1, and simian type-D retrovirus serogroups SRV-1, SRV-2, and SRV-5.



Fig. 1. Firm swelling on ventral midline of the abdomen cranial to umbilicus in an 8-year-old male rhesus macaque (*M. mulatta*).

Additional blood samples were taken for hematology. Two weeks after the surgical removal of the first mass, two additional small subcutaneous nodules, approximately 2.0 cm in diameter, were detected about 2.0 cm distal to the umbilicus on the ventral midline. Over the next 2 weeks, these two nodules coalesced into a single subcutaneous mass, approximately  $4.5 \times 4.0 \times 2.7$  cm in diameter. This second mass was surgically removed, as previously described excluding the laparotomy, and submitted for histologic examination.

Factor VIII-related antigen (F VIII-Rag) is an endothelial cell marker that has been documented in many animal species and humans [1, 13]. Three glass slides prepared from the tumor tissue samples of this rhesus monkey were stained for human F VIII-Rag (Novocastra Laboratories Ltd., Newcastle upon Tyne, NE12 8EW, UK). Normal blood vessels from the subcutaneous tissues of this monkey were embedded in paraffin wax and stained to serve as internal positive controls. A second tissue section was stained with phosphate-buffered saline to serve as a negative control.

## Results

The fine-needle aspirate consisted of numerous erythrocytes with a few polymorphonuclear cells and lymphocytes. One nonhematogenous cell had an elongated nucleus with prominent nucleoli. No bacteria were cultured from the sample submitted for microbial culture. Ventral–dorsal and lateral radiographs of this primate's chest and abdomen were unremarkable. Upon surgical removal of both masses, they appeared grossly as raised areas under the skin. During the resection procedure, both masses were completely surrounded by abdominal fat with no evidence of invasion into the abdominal musculature. The cut surface of the first mass showed a blood-filled hollow core encapsulated and surrounded by lobulated pale yellow abdominal fat (Fig. 2). Gross examination of the recurrent mass was identical to the initial mass. Histopathology of the biopsy samples taken from the blood-filled cores of both masses showed foci of spindle-shaped cells surrounding vascular spaces with no necrotic or inflammatory cell component (Fig. 3). Although gross examination of both masses showed no invasive characteristics, histologic examination of the tumor margins showed that there was abundant subcutaneous edema with fragmentation of collagen adjacent to the skin. Many spindle-shaped cells had prominent nucleoli (Fig. 4); some cells contained mitotic figures. There was definite pleomorphism of the cells lining the vascular channels. Numerous cells were multinu-

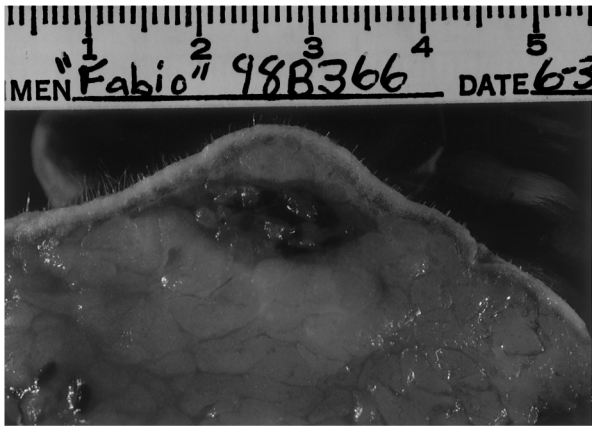


Fig. 2. Cross-section of mass, the cut surface is pale yellow in color with a blood-filled hollow core that consisted spindle-shaped cells surrounded by vascular spaces.

cleated; one cell exhibited anisokaryosis and marked variation in nucleolar size with nucleolar prominence. The cells were interspersed in connective tissue ground substance with foci of basophilia. The tumors had greater than three nuclear criteria of malignancy with no inflammatory cell (leukocyte, monocyte) component [2]. Based on the histopathologic evaluation, we classified the tumors evaluated in this monkey as malignant. An exploratory laparotomy, performed after the removal of the first mass, showed no gross lesions of the spleen, liver, kidneys, or gastrointestinal tract. Serum samples submitted from this primate were negative for antibodies to Lentivirus (SIV), STLV-1, simian type-D retrovirus serogroups SRV-1, SRV-2, and SRV-5. Hematology results from blood samples taken from the monkey during clinical presentation of both masses and 14 months after surgical removal were unremarkable. The pleomorphic cells lining the vascular channels of

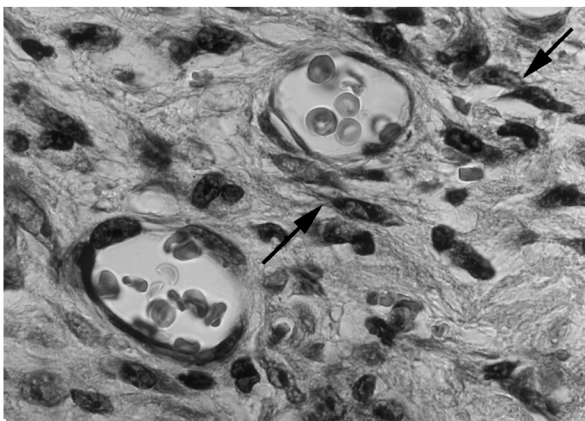


Fig. 3. Photomicrograph (100 ×) of H/E stained mid section of the mass showing foci of spindle-shaped cells surrounding vascular spaces (arrows).

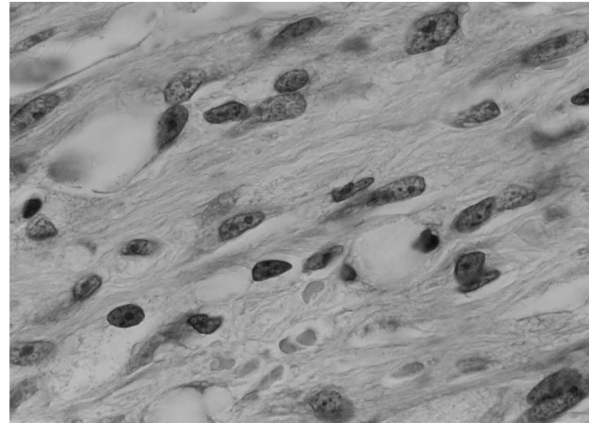


Fig. 4. Photomicrograph (100 ×) of H/E stained section of the mass showing many spindle-shaped cells with prominent nucleoli.

the tumor stained positive for F VIII-Rag, as did the vascular endothelial cells of both the subcutaneous venules and arterioles (controls). There was also multifocal staining of neoplastic cells in the surrounding connective tissue matrix (Fig. 5). Positive staining for F VIII-Rag confirmed that the neoplastic cells were endothelial in origin, consistent with hemangiosarcoma.

#### Discussion

In this case report, we have described recurrent hemangiosarcoma in an adult rhesus macaque (*M. mulatta*). Hemangiosarcoma has been reported in a number of different animal species but has rarely been documented in nonhuman primates. It has historically been an aggressive metastatic tumor of endothelial cell origin that is contiguous with the

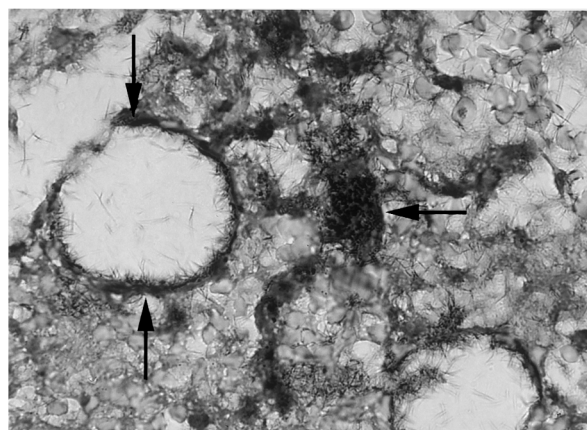


Fig. 5. Photomicrograph (100 ×) of mass showing positive F VIII-Rag staining of neoplastic cells in the connective tissue matrix and endothelial cells lining vascular channels. Positive F VIII-Rag staining stained neoplastic cells true black (arrows).

blood vascular system [2]. As the initial tumor progressed, a fine-needle aspirate was performed. Fine-needle aspiration is a common diagnostic technique used in veterinary oncology. However, this technique has been reported to have a decreased sensitivity in the assessment of hemangiosarcomas in dogs and cats, and such an aspirate also carries a risk of tumor seeding and hemorrhage [1]. On the other hand, cytologic diagnosis has proven to be a useful tool in evaluating epithelial and mesenchymal tumors of the skin in nonhuman primates [15]. Aspirates from hemangiosarcomas usually yield blood and a few endothelial cells [2]. In this case, the fine-needle aspirate did include one cell with prominent nucleoli and an elongated nucleus.

In this monkey, the tumors were confined to the subcutaneous tissue of the ventral abdomen and were removed easily by wide excisional resection. Dermal and subcutaneous hemangiosarcomas have been documented in dogs and cats and have been successfully managed by aggressive wide surgical resection of the tumor [1, 13, 14]. In cases of soft tissue hemangiosarcomas (angiosarcomas) in humans, it is recommended that complete excision of all gross tumor, its surrounding capsule, and resection of adjacent organs be performed in order to maximize the chance of removing all microscopic cancer cells [6, 7, 11]. However, wide surgical resection of a tumor may not totally eliminate the possibility of local recurrence or metastasis of the tumor. To date, surgical excision has successfully eliminated the need for additional treatments in this monkey. As with human patients, the management of nonhuman primates with neoplasia may require traditional or experimental anticancer treatments following surgical removal of the tumor [15].

Immunohistochemical evaluation of poorly differentiated mesenchymal tumors has proven to be a useful diagnostic tool to determine cellular origin of specific tumors. F VIII-Rag is normally synthesized by endothelial cells, platelets, and megakaryocytes. Specific tumor types including hemangiosarcomas, hemangiomas, and Kaposi's sarcomas also synthesize it. Tissue sections from the masses removed from this rhesus monkey stained positive for F VIII-Rag, confirming that the neoplastic cells were of endothelial origin. However, an immunohistochemical stain of a tumor known to be of endothelial origin may, on occasion, show a negative result. Negative staining results of this type can be attributed to either a F VIII-Rag deficiency (von Willebrand's disease), poor tissue preservation, or technical error during the staining process [13].

In conclusion, both masses removed from this rhesus monkey were consistent with hemangiosarcoma. This monkey is currently participating in an approved research protocol and is in sound health and physical condition. To our knowledge, this is the first report of recurrent subcutaneous hemangiosarcoma in a rhesus monkey.

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