Case Report

Hepatic Hemangiosarcoma in a Cynomolgus Macaque (*Macaca fascicularis*)

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An experimentally naïve, 9-y-old, intact male cynomolgus macaque was reported for bleeding from an unidentified site. Sedated physical examination indicated mild gingival separation from the lingual aspect of the upper right canine tooth as the source of the hemorrhage. Physical exam revealed a firm mass adhered to the left zygomatic arch, 2 subcutaneous nodules on the chest, and a large mass in the cranial abdomen. Radiographs revealed a large soft-tissue mass in the cranial abdomen and multifocal nodules in the caudal lung fields. On ultrasonography, the liver was grossly enlarged and contained a cavitated mass. Hematology and serum chemistry results demonstrated severe regenerative anemia with normal clotting times and adequate platelet count. For humane reasons, euthanasia was elected. On gross examination, the liver was markedly enlarged by an expansile mass primarily affecting the median lobe, with multiple, smaller nodules throughout the remaining lobes. Multifocal round, firm nodules were observed on the surface of all lung lobes and throughout the omentum. Histologic examination of the hepatic, cutaneous, osseous, and pulmonary lesions demonstrated well-defined, endothelium-lined vascular channels arranged in cords with abundant hemorrhage; endothelial-cell immunomarkers confirmed these results. On the basis of these findings, hepatic hemangiosarcoma, with metastases to the lungs, omentum, subcutis, and bone, was diagnosed. This case study is the first report of spontaneous hepatic hemangiosarcoma in a cynomolgus macaque and the first case with metastasis to bone in a NHP.

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Hemangiosarcoma is a malignant neoplasm of endothelial cell origin that forms aberrant vascular channels; and this tumor has intermittently been reported to affect NHP.^{4,7,15,16,25,27,41} In humans, hemangiosarcoma (also known as angiosarcoma) is a rare but aggressive tumor, with a prevalence of approximately 2 million (fewer than 600 cases reported each year).⁹ Among all species, this neoplasm is most commonly reported in canines, with spontaneous hemangiosarcoma representing approximately 5% to 7% of all canine malignant tumors.³⁸ The current veterinary staging system applied to canine and feline patients is the modified Tumor–Node–Metastasis (TNM) World Health Organization staging protocol,^{2,33} where stage I is a tumor confined to the primary organ without metastasis, stage II includes a tumor confined to the primary organ or ruptured, with or without lymph node involvement but without distant metastasis, and stage III involves distant metastasis.²¹

Hemangiosarcoma is a very aggressive neoplasm, with a survival rate of less than 10% at 1 y after diagnosis in canines.³⁵ Depending on the stage and organ(s) affected, the primary method of treatment is surgery with adjuvant chemotherapy.^{28,42} Chemotherapeutic agents used for treatment in veterinary patients have included doxorubicin or epirubicin alone²⁸ and

doxorubicin combined with ifosfamide,³⁰ dacarbazine,¹³ vincristine and cyclophosphamide,^{6,17,37} or dacarbazine and vincristine.¹⁰ Chemotherapy can delay metastasis and prolong survival time, but many of the aforementioned protocols are associated with various hematologic and gastrointestinal toxicities and are most effective for stage I or II hemangiosarcoma.¹³

Hepatic angiosarcoma is rare in humans, comprising less than 1% of all sarcomas²³ and only approximately 2% of hepatic malignancies.^{23,27} Hepatic angiosarcoma in humans used to have a high fatality rate—in the 1970s, only 3% of patients survived longer than 2 y. However a recent 10-y retrospective analysis reports that 2-y survival rates for this patient subgroup have improved to as high as 33% after a combination of surgery and adjuvant chemotherapy.¹⁸ In NHP, only a single case of hemangiosarcoma has definitively been reported to be of hepatic origin, and this lesion was in a juvenile rhesus macaque.²⁵ To our knowledge, our current case report is the first description of hepatic hemangiosarcoma in a cynomolgus macaque and the first report of this neoplasm's metastasis to bone in NHP.

Case Report

An experimentally naïve, 9-y-old intact male cynomolgus macaque (*Macaca fascicularis*) was reported by the animal care staff for bleeding from an unknown origin. Cage-side assessment revealed that the patient had lost 5 to 10 mL of blood, which was pooled on the floor of the primary enclosure. He exhibited no visible outward signs of injury and was singly housed at the time. The macaque was sedated with ketamine (4 mg/kg IM) and dexmedetomidine (0.05 mg/kg IM) for examination and further diagnostics.

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All animal care, use, and husbandry practices conformed to standards established by the Animal Welfare Act,¹ AAALAC International, and the *Guide for the Care and Use of Laboratory Animals*.¹⁹ All procedures for care followed the guidelines of the University of Pennsylvania IACUC, and the macaque was on an IACUC-approved protocol. He was SPF for *Macacine herpesvirus 1*, simian retroviruses (SRV1, SRV2, SIV, and simian T-cell leukemia virus type 1), and *Mycobacterium tuberculosis* complex. The macaque was housed indoors, fed standard chow (Monkey Diet 5038, LabDiet, St Louis, MO), and provided water without restriction and daily dietary and environmental enrichment.

On physical exam, vital signs were within normal limits (99.6 °F; heart rate, 60 to 90 bpm; respiratory rate, 24 breaths per minute), and the macaque's body condition score was normal (6 on a scale of 9). Mild gingival separation and a pinpoint ulcer were present at the level of the right maxillary canine. Hemostasis was not achieved after 5 to 7 min of digital pressure, so epinephrine was applied topically in conjunction with manual pressure until clotting was noted. A firm mass (3.0 × 4.0 × 3.0 cm) was palpated contiguous with the left zygomatic arch. A large, firm mass was palpable within the cranial abdomen. Two raised, erythematous, subcutaneous nodules (diameter, 1.0 to 2.0 cm) were present on the left and right chest.

During examination, 8 mL of blood was sampled from a peripheral vein for a CBC, hematologic chemistry profile, and coagulation panel. The CBC indicated severe macrocytic, normochromic, regenerative anemia (RBC: $3.1 \times 10^6/\mu\text{L}$; reference range, 6.31 to $7.55 \times 10^6/\mu\text{L}$), Hct: 23%; reference range, 42.0% to 49.2%). Results of the blood chemistry profile and coagulation panel (prothrombin time, partial thromboplastin time, fibrinogen, D-dimer) were within normal limits.

Radiographs of the skull revealed a lytic, osseous mass of the left zygomatic arch with moderate periosteal reaction (Figure 1). Thoracic and abdominal radiographs showed the presence of multiple pulmonary nodules (diameter, 0.5 to 1.0 cm; Figure 2 A and B), as well as a large cranial abdominal mass that caudally displaced the small and large intestines. Ultrasonographic imaging showed hepatomegaly, with a large (diameter, 4.0 to 6.0 cm) hypoechoic, cavitated mass in the caudal liver.

Due to the guarded to poor prognosis associated with suspected neoplasia with metastases to the lungs, euthanasia was elected, and a necropsy was performed. Consistent with the findings from external physical examination previously, we noted 2 round, raised, erythematous subcutaneous nodules on the ventral chest (right, 1.0 cm; left, 2.0 cm; Figure 3). The osseous mass on the left zygomatic arch (3.0 × 4.0 × 3.0 cm) was readily visible after shaving of the facial hair (Figure 3). Within the peritoneal cavity, there were multiple fibrous adhesions between the mesentery and parietal peritoneum. Within the omentum were multifocal to coalescing, 0.25 to 0.5-cm, dark red, firm, spherical nodules. The liver contained a large (10.5 × 5.9 × 7.5 cm) expansile mass that replaced the median and caudate lobes (Figure 3). On cut surface, the mass was multilobular and cavitated, with a soft, dark red center. There was a singular, cavitated nodule in the right liver lobe (3.0 × 3.0 × 1.8 cm). In the left liver lobe was a subcapsular, fluid-filled, raised cystic lesion (1.8 × 2.4 × 0.4 cm). On the pleural surface, and extending into the pulmonary parenchyma on cross section, were multiple, round, dark, firm masses ranging from 0.5 to 2.0 cm in diameter (Figure 3).

Tissue samples from the lesions were fixed in 10% buffered formalin, processed routinely for histologic sectioning, cut at 5.0 μm , and then stained with hematoxylin and eosin. On histopathologic analysis, the parenchyma of the median liver lobe

Figure 1. Ventrodorsal view of the cranium of the cynomolgus macaque. Note the expansile, lytic mass on the left zygomatic arch.

was diffusely expanded, effaced, and replaced by a nonencapsulated, poorly demarcated, infiltrative mass. The mass was composed of spindloid mesenchymal cells forming variably sized, endothelium-lined, blood-filled vascular channels (Figure 4). Neoplastic cells were flattened to plump, with indistinct margins, scant eosinophilic, granular cytoplasm, and oval nuclei with coarsely clumped chromatin and indistinct nucleoli. There was moderate pleomorphism and anisokaryosis, with 10 to 15 mitotic figures noted per high-power field. There was multifocal, marked hemorrhage throughout the mass. The histologic architecture of the lung, zygomatic arch bone, and subcutis were extensively effaced by neoplastic nodules that were similar in morphology to the hepatic lesions (Figure 4).

To further characterize the neoplasm, immunohistochemistry was performed for biomarkers commonly overexpressed in hemangiosarcoma. For indirect immunohistochemistry, 5- μm serial sections on negatively charged glass slides were obtained from formalin-fixed paraffin-embedded tissues. The IntelliPATH staining system (Biocare Medical, Pacheco, CA) was used for immunostaining. The following primary antibodies were applied to sections for 30 min at room temperature: CD31 (1:20 dilution; catalog no. M0823, mouse monoclonal, Dako, Agilent, Santa Clara, CA), vimentin (1:50 dilution; catalog no. M0725, mouse monoclonal, Dako), and factor VIII-related antigen (1:1000 dilution; catalog no. A0082, rabbit polyclonal, Dako). Immunodetection for CD31 and vimentin was performed by using a Dako EnVision kit (Agilent) containing an ENVmono-HRP-labeled polymer-conjugated mouse secondary antibody. Immunodetection for factor VIII-related antigen was performed by using a Dako LSAB2 kit (Agilent). Visualization of antibody binding was obtained by using the chromogen 3,3'-diaminobenzidine. Sample tissues were incubated with Tris-buffered saline substituted for the primary antibody as a negative control.

The neoplastic, spindloid cells from the 4 affected sites examined (liver, lungs, zygomatic arch, and skin) exhibited strong positive cytoplasmic staining for CD31 (Figure 5 A through D). Strong, positive cytoplasmic staining for vimentin was observed diffusely in all neoplastic cells (Figure 6 A through D), with no off-target labeling of normal tissue adjacent to the neoplasms. Moderate to sporadic cytoplasmic staining for factor VIII-related antigen was observed (Figure 7 A through D), with the greatest intensity localized to the endothelial cells lining the osteoid-effacing vascular channels (Figure 7 C). Normal endothelial cells in unaffected regions within the histologic sections

Figure 2. (A) Left lateral thoracic and (B) ventrodorsal radiographs reveal multifocal pulmonary nodules, which are most prominent in the caudoventral lung fields (arrows).

labeled appropriately positively with CD31, vimentin, and factor VIII-related antigen, thus serving as internal positive controls; positive controls from NHP small intestine samples also labeled appropriately positively with the 3 immunomarkers.

Discussion

Vascular tumors such as hemangiosarcoma are rare in macaques, with only a few examples reported in the scientific literature. Hemangiosarcoma has been well documented in other species, including humans and dogs. In NHP, hemangiosarcoma has been reported sporadically in a variety of species: tamarins (renal hemangiosarcoma,¹⁶ urinary bladder hemangiosarcoma),¹⁵ a spider monkey (uterine hemangiosarcoma),⁷ and rhesus macaques (subcutaneous hemangiosarcoma,²⁶ epithelioid hemangiosarcoma).⁴¹

To our knowledge, this current report is the first description of primary hepatic hemangiosarcoma in a cynomolgus macaque. Before the current case, the only previous report of hemangiosarcoma in a cynomolgus macaque described a mass of subcutaneous and splenic origin with metastases to the muscles, liver, and pleural cavity.⁵ Only one other case of primary hepatic hemangiosarcoma has been reported in an NHP. In that case, a rhesus macaque had a single large primary tumor and multiple smaller masses, all located within the liver, with no metastases.²⁵ In contrast, in our case, the primary tumor had metastasized to multiple organs, including omentum, lungs, skin, and bone, by the time of diagnosis. To our knowledge, this macaque also represents the first case report of hemangiosarcoma that has metastasized to bone in an NHP.

A histologic diagnosis of hemangiosarcoma was made after observing the spindloid mesenchymal cells forming endothelium-lined vascular channels accompanied by hemorrhage, which are characteristic of this neoplasm. Extrapolating from the World Health Organization veterinary staging system for companion animals, the neoplasm in this macaque, with its distant metastasis, would be considered a stage III hemangiosarcoma.²¹

Our diagnosis of presumptive hepatic neoplasia was based on a combination of physical exam, radiographic, ultrasonographic, and hematologic findings. Diagnosis of hemangiosarcoma was not confirmed until results from histopathology were finalized (a combination of hematoxylin and eosin staining and immunohistochemistry for endothelial-specific markers). Had treatment been considered for this animal, the neoplasm would likely have been characterized through histopathology of liver biopsy samples (open, endoscopic, or transcutaneous), with staging for extent of local invasiveness and metastases determined by portal phase-enhanced CT or MRI.³¹ Currently, radical surgical resection is considered the treatment of choice for primary hepatic angiosarcoma in humans.⁴⁰ In one study of 9 patients who underwent hepatic resection for primary hepatic angiosarcoma, median overall survival and disease-free survival were 59 and 11 mo, respectively.⁴⁰ However, the tumors in all 9 patients were histologic grade II, meaning that a patient who had metastases similar to the animal we described here (that is, grade III) would be a poor candidate for surgical resection. For advanced disease, chemotherapy currently offers minimal benefit in terms of survival time, with a few exceptions. A study of human patients receiving a combination of

Figure 3. Postmortem examination findings. (A) Two raised subcutaneous nodules (1.0 cm, 2.0 cm) near shoulders (arrows). (B) Zygomatic arch mass (3.0 × 4.0 × 3.0 cm) visible after shaving. (C) Liver contained a neoplastic mass (10.5 × 5.9 × 7.5 cm) replacing the median lobe; a singular, cavitated nodule in the right lobe (3.0 × 3.0 × 1.8 cm; dashed arrow); a subcapsular, fluid-filled, raised lesion (1.8 × 2.4 × 0.4 cm) in the left lobe (arrow). (D) Lungs contain multiple dark, firm nodules (0.5 to 2.0 cm).

doxorubicin–carboplatin and 5-fluorouracil saw survival times of 9 and 16 mo in 2 patients.³ In a second case series, palliative chemotherapy using a combination of amycin, cisplatin, ifosamid, and paclitaxel achieved greater than 14 mo of survival after diagnosis in 2 patients.²²

For canine patients, which comprise the majority of veterinary hemangiosarcoma cases in the literature, the prognosis is grim. With surgical excision and adjuvant chemotherapy, the average survival time for splenic hemangiosarcoma is 3 to 6 mo, and with surgery alone, just 1 to 3 mo.⁴³ In addition to surgical resection and traditional chemotherapeutic approaches, new techniques including laparoscopic microwave ablation of liver neoplasms have been reported in a small subset of patients.²⁹ A recent study demonstrated that administration of the peroxisome proliferator activated receptor α agonist fenofibrate provided increased survival time (as long as 26 mo) after splenectomy in a canine hemangiosarcoma patient.^{12A}

recent study in 104 canine patients with hemangiosarcoma indicated that administration of blood products and the presence of gross metastatic disease at the time of surgery shortened the disease-free interval, whereas administration of the Chinese herbal supplement Yunnan Baiyao prolonged the disease-free interval.⁸

To confirm findings, immunohistochemistry was performed using endothelial cell immunomarkers that were selected due to their widely-accepted use in the diagnosis of hemangiosarcoma. CD31, also known as PECAM1 (platelet endothelial cell adhesion molecule 1), is a glycoprotein that is expressed on endothelial cells, platelets, and leukocytes and is involved in many physiologic and pathologic pathways, including leukocyte migration, angiogenesis, maintenance of vascular integrity, and immunoregulatory roles in endothelial and hematopoietic cells.^{11,32,39} CD31 is positively expressed in vascular neoplasms of many species, including dogs, cats, NHP, and humans,^{7,20,32,41}

Figure 4. (A) Liver, hemangiosarcoma. Neoplastic cells are arranged in cords, forming blood-filled, vascular channels. The parenchyma is diffusely expanded, effaced, and replaced by hemangiosarcoma. (B) Liver, hemangiosarcoma. Plump spindloid neoplastic cells line vascular channels that contain RBC. (C) Lung, hemangiosarcoma. (D) Skin, hemangiosarcoma. (E) Bone, zygomatic arch, hemangiosarcoma. (F) Bone, zygomatic arch, hemangiosarcoma. Hematoxylin and eosin stain; magnification: 2 $\times$ (A, C, D, E); 10 $\times$ (F); 40 $\times$ (B).

and the tumor in our case exhibited strong positive cytoplasmic staining. Vimentin is a filamentous protein that is expressed on normal mesenchymal cells and plays a role in maintenance of cellular integrity.³⁴ Positive staining for vimentin indicates neoplastic cells of mesenchymal origin.³⁶ As with factor VIII-related antigen, a majority of human cases of hemangiosarcoma stain

positively for vimentin.²⁴ The tumor in this case also stained positively for vimentin. Factor VIII-related antigen (also known as von Willibrand factor), a component of the factor VIII complex, is a glycoprotein expressed on endothelial cells, platelets, and megakaryocytes.⁴ Factor VIII-related antigen is an endothelial cell marker used in many species and is a well-documented

Figure 5. Immunohistochemistry for CD31 in (A) liver, (B) lungs, (C) bone, and (D) skin. Strong positive labeling of the endothelial cell cytoplasm is present.

ancillary test for hemangiosarcoma diagnosis.^{4,14} The current case demonstrated moderate to sporadic positive cytoplasmic staining. Together, these immunohistochemistry findings confirm our histologic findings and support our diagnosis of hemangiosarcoma.

In summary, the current report describes a case of spontaneous hepatic hemangiosarcoma with metastases to the subcutis, lungs, omentum, and bone in a cynomolgus macaque. The diagnosis of hemangiosarcoma is consistent with the observation of spindloid mesenchymal cells that formed endothelial channels and positive staining for 3 commonly used hemangiosarcoma immunomarkers: CD31, vimentin, and factor VIII-related antigen. To our knowledge, this case of hepatic hemangiosarcoma is the first to be described in a cynomolgus macaque, and the first case of hemangiosarcoma with metastasis to the bone in an NHP.

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contributed equally to this work and consider first authorship to be interchangeable.

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Figure 6. Immunohistochemistry for vimentin in (A) liver, (B) lungs, (C) bone, and (D) skin. Strong, positive, diffuse labeling of neoplastic cells is present in all 4 locations.

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Figure 7. Immunohistochemistry for factor VIII-related antigen in (A) liver, (B) lungs, (C) bone, and (D) skin. Moderate to sporadic cytoplasmic staining for factor VIII-related antigen is present, with the greatest intensity in the osteoid-effacing neoplasm (C).

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