Published in final edited form as:

J Med Primatol. 2010 December ; 39(6): 385–388. doi:10.1111/j.1600-0684.2010.00425.x.

Splenic Angioleiomyoma in an Owl monkey (*Aotus nancymae*)

Alfonso S Gozalo^{1,3}, Patricia M Zervas², Matthew F Starost², William R Elkins¹, and Carol L Clarke¹

¹Comparative Medicine Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

²Division of Veterinary Resources, Office of the Director, National Institutes of Health, Bethesda, MD

³SoBran Inc., Bethesda, MD

Abstract

Background—An adult male owl monkey (*Aotus nancymae*) underwent a splenectomy. When the spleen was removed, a small, nodular mass slightly bulging over the splenic surface was noted.

Methods—The mass was examined by light and transmission electron microscopy and by immunohistochemistry.

Results—On light microscopy, the mass was well circumscribed, unencapsulated, and composed of haphazardly arranged smooth muscle bundles admixed with numerous small capillary-like structures containing blood. Immunohistochemical (IHC) staining revealed the tumor was strongly positive for smooth muscle actin yielding vascular smooth muscle bundles, and to Factor VIII, staining endothelial cells within the smooth muscle bundles. Transmission electron microscopy (TEM) showed a large portion of the cells to be atypical appearing smooth muscle and a few cells had structures resembling Weibel-Palade bodies indicating endothelial cells.

Conclusions—Based on cell morphology, by light and TEM, and IHC a final diagnosis of splenic angioleiomyoma was made. This is, to our knowledge, the first report of an angioleiomyoma in a nonhuman primate.

Keywords

Cebidae; immunohistochemistry; neoplasia; nonhuman primate; spleen; transmission electron microscopy; vascular leiomyoma

Introduction

An angioleiomyoma or vascular leiomyoma is a rare benign neoplasm composed of thick smooth muscle walled-vessels. The tumor originates in the tunica media of veins and can be divided into 3 subtypes: capillary or solid, venous, and cavernous [3]. Capillary or solid type is the most common of the three characterized by closely compact smooth muscle and small,

Corresponding author: Alfonso Gozalo, DVM, MS, DACLAM, Comparative Medicine Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, SoBran Inc./Government Contractor, 9000 Rockville Pike, Building 14B South, Room 228, Bethesda, Maryland 20892, Tel: 301-496-9333, Fax: 301-480-2377, gozaloa@niaid.nih.gov.

Author Contributions:

AG, MS Designed the study

MS, PZ Performed the experiments

AG, MS, PZ, CC Analyzed the data

AG, PZ, MS, WE, CC Wrote the manuscript

slit-like, vascular channels. In humans, this tumor is more common in females than males. The venous type is characteristic by thick muscular walls and is more common in males. Cavernous type, which is the least common, occurs more in males and contains dilated vascular channels and few smooth muscle cells. Angioleiomyomas have a peak incidence in humans between 30 and 60 years of age and present as a solitary nodule which may or may not be painful [3,10]. The tumor is usually found in the extremities, particularly the lower leg, although there have been reports of other locations such as the oral cavity [2], external auditory canal [14], lacrimal sac [7], uterus [6], mediastinum [5], knee joint [13], and intracranially [8]. Angioleiomyomas have also been described in the dog and the cat on the distal extremities [1,9]. Surgery is usually curative for man and animals; recurrence has been documented in the dog [1]. To our knowledge, this is the first report of an angioleiomyoma in a nonhuman primate.

Case Report

In 2009, a 1 kg, 11-year old, wild caught, male, owl monkey (*Aotus nancymae*) underwent an experimental splenectomy as part of an Institutional Animal Care and Use Committee-approved malaria vaccine study. The animal was housed under a 12:12-h reverse light cycle (dark from 1 pm to 1 am with no red lighting used), at a room temperature of 18–29°C, with a relative humidity of 30–70% in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals [11]. Water was provided by water bottles and the diet consisted of chow (Primate Diet 8794N, Harlan Teklad, Madison, WI), fruits, and treats (Bio-Serv, Frenchtown, NJ). The animal was tested semi-annually for tuberculosis and found to be negative. The previous history of the animal was that in 2008 it was diagnosed with a cardiomyopathy and placed on furosemide (0.1 mL IM once daily), enalapril (1 mg PO twice daily), and spironolactone (25 mg PO twice daily). Later it was placed on glipizide (1.5 mg PO twice daily) for hyperglycemia and the furosemide was discontinued. In 2004 the animal received *Plasmodium falciparum* [FVO-CDC (2937)] by intravenous injection and was cured with 25 mg of mefloquine PO on a single dose two weeks later.

At surgery, when the spleen was removed, a small, approximately 5 mm, nodular mass slightly bulging over the spleen surface was noted. The spleen was fixed in 10% neutral buffered formalin, processed, embedded in paraffin, sections cut at 5 µm and stained with hematoxylineosin. Immunohistochemistry for smooth muscle actin and factor VIII was performed on the mass. Briefly, four micron sections were deparaffinized in xylene, placed in absolute alcohol, and processed in a DAKO Autostainer Universal Staining System (DAKO, Carpinteria, CA). Endogenous peroxidase was inhibited by 5-minute incubation in 3% hydrogen peroxide. Antigen retrieval was accomplished with pepsin for 15 minutes (Factor VIII) at room temperature. Sections were blocked with a commercially available protein block (Dako) for 5 minutes at room temperature. Mouse anti-factor VIII (human von Willebrand Factor; species reactivity-human, mouse, and rat; 1:500; Biocare Medical, Concord, CA) and mouse anti-smooth muscle actin (clone 1A4; species reactivity-human, mouse, and rat; 1:100; Biocare Medical) were the primary antibodies. The detection system utilized a polymerized HRP anti-mouse secondary antibody for both primary antibodies. DAB chromogen (DAKO) was added and the slides counterstained with Mayer's hematoxylin. Positive control slides consisted of known positive mammalian tissue incubated with the relevant monoclonal antibody. Negative control slides consisted of the test tissue incubated with an isotype matched immunoglobulin. An interpretation of positive staining was based on the presence of either strong, diffuse cytoplasmic staining or patchy cytoplasmic staining of varying intensity. For transmission electron microscopy (TEM), because the tumor was very small and no tumor samples were left in formalin, the area of interest was trimmed from the paraffin block, washed three times in xylene for one hour each and placed into 10% OsO₄ in xylene overnight. Subsequently the tissue was washed

three times in xylene for 30 mins each, changed to 2:1 propylene oxide: EPON, 1:2 propylene oxide: EPON, and embedded in EMBed 812 resin (Electron Microscopy Sciences, Hatfield, PA). Thin sections, approximately 80 nm, were obtained by utilizing a Leica ultracut-UCT ultramicrotome (Leica, Deerfield, IL) and placed onto 300 mesh copper grids and stained with saturated uranyl acetate in 50% methanol and then with lead citrate. The grids were viewed in a JEM-1200EXII electron microscope (JEOL Ltd, Tokyo, Japan) at 80kV and images were recorded on a XR611M, mid mounted, 10.5Mpixel, CCD camera (Advanced Microscopy Techniques Corp, Danvers, MA).

Results

On light microscopy, the mass was well circumscribed, unencapsulated, (Figure 1) and composed of haphazardly arranged smooth muscle bundles admixed with numerous small capillary-like structures containing blood (Figure 2). In some cases, the capillary-like lumina were very narrow, in others, wider and tortuous or stellate. Lining these structures were suspect endothelial cells that appeared thin and elongated in some cases while swollen and hypertrophied in others. In many instances erythrocytes appeared to be in the tumor interstitium with no endothelial cells surrounding them. Occasional mitotic figures were observed. IHC staining revealed the tumor was strongly positive to SMA yielding vascular smooth muscle bundles (Figure 3), and to Factor VIII, staining endothelial cells within the smooth muscle bundles. Factor VIII revealed endothelial cells to vary in shape from very narrow, with elongated nuclei, to epithelioid-like. TEM showed a large portion of the cells to be atypical appearing smooth muscle. The smooth muscle cells were densely packed with a variety of shapes ranging from rounded to spindle (Figure 4). The cytoplasm was prominently filled with intermediate filaments to such a degree that in places other organelles were displaced and the nuclei were disfigured. Few ribosomes, mitochondria, dilated cisternae and Golgi remained in the cytoplasm. The nucleus was centralized with invaginations displaying a prominent nucleolus. Red blood cells were observed in the interstitial spaces with no clear vascular channels. A few cells had cytoplasmic structures resembling Weibel-Palade bodies indicative of endothelial cells. Based on cell morphology by light and TEM, and IHC, a final diagnosis of splenic angioleiomyoma was made.

Discussion

The tumor in our case resembles the capillary or solid type of angioleiomyoma which is characterized by closely compact smooth muscle and small, slit-like, vascular channels [3]. The lesion was considered an incidental finding with no clinical significance. The histogenesis of angioleiomyomas is still debated. However, most authors agree that vascular leiomyomas arise from veins [3,4]. The most common feature of these tumors on histologic examination is bundles of mature smooth muscles oriented around blood vessels. To date, the literature for man and animal does not cite the induction of angioleiomyomas by *Plasmodium* or by any of the medications the animal received. Interestingly, splenic nodules resembling tumors have been described in wild-caught cynomolgus monkeys naturally infected with the nematode *Edesonfilaria malayensis*. Histologically, the splenic lesions are characterized by well-circumscribed fibrotic areas with accumulation of inflammatory cells and intimal thickening of small arteries with presence of *E. malayensis* microfilariae [12]. No inflammatory cells or microfilariae were noted in the splenic tumor in our case. This is, to our knowledge, the first report of an angioleiomyoma in a nonhuman primate.

Acknowledgments

This study was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Allergy and Infectious Diseases, Comparative Medicine Branch, Laboratory of Malaria Immunology and Vaccinology, the Office of Research Services, and a NIAID contract to SoBran Inc.

References

1. vallone G, Helmbold P, Caniatti M, Stefanello D, Nayak RC, Roccabianca P. The spectrum of canine cutaneous perivascular wall tumors: morphologic, phenotypic, and clinical characterization. *Vet Pathol.* 2007; 44(5):607–620. [PubMed: 17846233]
2. Brooks JK, Nikitakis NG, Goodman NJ, Levy BA. Clinicopathologic characterization of oral angioleiomyomas. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2002; 94(2):221–227. [PubMed: 12221390]
3. Gomez-Dermitt V, Gallardo E, Landeras L, Echevarria F, Barredo R. Subcutaneous angioleiomyomas: Gray-scale and color Doppler sonographic appearances. *J Clin Ultrasound.* 2006; 34(2):50–54. [PubMed: 16547985]
4. Hachisuga T, Hashimoto H, Enjoji M. Angioleiomyoma. A clinicopathologic reappraisal of 562 cases. *Cancer.* 1984; 54(1):126–130. [PubMed: 6722737]
5. Hoseok I, Jeong YJ, Choi KU, Kim YD. Symptomatic posterior mediastinal angioleiomyoma. *Yonsei Med J.* 2008; 49(4):666–668. [PubMed: 18729312]
6. Hsieh CH, Lui CC, Huang SC, Ou YC, ChangChien CC, Lan KC, Huang KH. Multiple uterine angioleiomyomas in a woman presenting with severe menorrhagia. *Gynecol Oncol.* 2003; 90(2): 348–352. [PubMed: 12893198]
7. Korn BS, Glasgow BJ, Kikkawa DO. Epiphora as a presenting sign of angioleiomyoma of the lacrimal sac. *Ophthal Plast Reconstr Surg.* 2007; 23(6):490–492.
8. Lach B, Duncan E, Rippstein P, Benoit BG. Primary intracranial pleomorphic angioleiomyoma--a new morphologic variant. An immunohistochemical and electron microscopic study. *Cancer.* 1994; 74(7):1915–1920. [PubMed: 8082097]
9. Liu SM, Mikaelian I. Cutaneous smooth muscle tumors in the Dog and Cat. *Vet Pathol.* 2003; 40(6): 685–692. [PubMed: 14608022]
10. Nagata S, Nishimura H, Uchida M, Hayabuchi N, Zenmyou M, Fukahori S. Giant angioleiomyoma in extremity: report of two cases. *Magn Reson Med Sci.* 2006; 5(2):113–118. [PubMed: 17008768]
11. National Research Council. Guide for the Care and Use of Laboratory Animals. Washington (DC): National Academy Press; 1996.
12. Nonoyama T, Sugitani, Orita S, Miyajima H. A pathological study in cynomolgus monkeys infected with *Edesonfilaria malayensis*. *Lab Anim Sci.* 1984; 34(6):604–609. [PubMed: 6521429]
13. Okahashi K, Sugimoto K, Iwai M, Oshima M, Takakura Y. Intra-articular angioleiomyoma of the knee: A case report. *Knee.* 2006; 13(4):330–332. [PubMed: 16647261]
14. Picciotti P, Cantore I, La Greca C, Di Nardo W, Scarano E. Angioleiomyoma of the external auditory canal. *Am J Otolaryngol.* 2007; 28(4):235–237. [PubMed: 17606037]

Figure 1.
Aotus nancymae. Spleen. Angioleiomyoma. Well circumscribed non-encapsulated mass within the splenic parenchyma. H&E. $\times 15$.

Figure 2.
Aotus nancymae. Spleen. Angioleiomyoma. Higher magnification of nodular mass showing smooth muscle bundles admixed with numerous small capillary-like structures containing blood. H&E. ×400.

Figure 3.
Aotus nancymae. Spleen. Angioleiomyoma. The neoplastic cells stain strongly for smooth muscle actin. Streptavidin-biotin immunoperoxidase stain. Hematoxylin counter stain. $\times 400$.

Figure 4. *Aotus nancymae*. Spleen. Angioleiomyoma. TEM showing elongated to oval cells with a central nuclei, and thin cytoplasmic filaments indicative of smooth muscle. Uranyl acetate and lead citrate stain. $\times 3000$. Bar = 2 microns.