

Case Report

Spontaneous Rhabdomyosarcoma in a Common Marmoset (*Callithrix jacchus*)

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Abstract: The common marmoset (*Callithrix jacchus*) is now widely used in various research fields, including toxicology. However, information about the background pathology of this species is scarce. Here, we report a case of rhabdomyosarcoma that spontaneously occurred in a common marmoset. A 44-month-old male common marmoset was euthanized due to bilateral hind limb paralysis. At necropsy, a 2×2×5-cm intramuscular mass was observed in the lower right back. Histologically, the mass was mainly composed of interlacing bundles of spindle-shaped tumor cells. Immunohistochemically, the tumor cells were positive for myogenin, desmin, vimentin and alpha-smooth muscle actin. Ultrastructurally, the tumor cells contained bundles of myofilaments with Z-band-like structures. Thus, the tumor was diagnosed as a rhabdomyosarcoma. To our knowledge, this is the first report of spontaneous rhabdomyosarcoma that was definitely diagnosed in the common marmoset. (DOI: 10.1293/tox.26.187; J Toxicol Pathol 2013; 26: 187–191)

Key words: *Callithrix jacchus*, immunohistochemistry, pathology, rhabdomyosarcoma

The common marmoset (*Callithrix jacchus*) is a New World primate. With the benefits of its high fertility rate and small size (250–500 grams in adulthood), the common marmoset is now widely used in various research fields, including toxicology¹. However, information about the background pathology of this species is scarce^{2,3}, and there have been only a few reports of spontaneous neoplasms, such as uterine adenocarcinoma⁴, small intestine carcinoma⁵⁻⁷, bronchial adenocarcinoma⁸ and nephroblastoma⁹.

Here, we report a case of rhabdomyosarcoma that spontaneously occurred in a common marmoset.

A facility-born, male common marmoset had been kept without drug administration. Procedures for animal care and housing were in compliance with the institutional guidelines for the care and use of laboratory animals. The animal was housed individually in a stainless steel cage (39 cm wide, 55 cm deep, 70 cm high) in an environmentally controlled room that provided a temperature range of 26–30°C, a relative humidity of 30-70%, fresh air at a rate of at least 10 changes/hour, and a light:dark cycle of 12:12 hours.

The marmoset was fed a commercial feed, CMS-1M (CLEA Japan, Inc., Tokyo, Japan), supplemented with honey, Vitamin C and Vitamin D. Municipal water was provided ad libitum via an automated system.

At 44 months of age, the animal abruptly showed paralysis of bilateral hind limbs and was euthanized by exsanguination via the abdominal aorta under deep pentobarbital anesthesia. At necropsy, a 2×2×5-cm intramuscular mass was observed in the lower right back (Fig. 1). The mass was widely encapsulated, but partly adhered to the lumbar vertebrae. The cut surface of the mass was soft and white (partly yellowish white). Several white nodules, below 5 mm in size, were found in the lung.

For histological examination, tissue samples of the primary mass as well as various organs, including the lung containing nodules, were fixed in neutral-buffered 10% formalin (peripheral lymph nodes were not sampled). Following fixation, the tissue samples were embedded in paraffin, sectioned at 3 µm and stained with hematoxylin and eosin (HE). Sections of the mass were also stained by Oil red-O and periodic acid-Schiff (PAS) methods. For the Oil red-O staining, frozen sections of the mass were used. For immunohistochemistry, sections of the mass were subjected to a labeled polymer method using Histofine Simple Stain Rat MAX PO (MULTI) (Nichirei Biosciences Inc., Tokyo, Japan). Primary antibodies used in this study are summarized in Table 1. For electron microscopic examination, small pieces of the mass fixed in neutral buffered 10% for-

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malin were refixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in Epon resin. Ultra-thin sections of the selected areas were prepared, contrasted with hafnium chloride and lead citrate, and examined using a JEM-1400 transmission electron microscope (JEOL Ltd., Tokyo, Japan).

Histologically, the mass was surrounded by the fascia with atrophic muscle fibers (Fig. 2A). The center of the mass was necrotic. The mass was mainly composed of interlacing bundles of spindle-shaped tumor cells (Fig. 2B). These tumor cells had elongated, blunt-ended nuclei and weakly basophilic, vaguely outlined cytoplasm (Fig. 2C). There were 6 to 10 mitotic figures per high-power field (400 ×). Some tumor cells had abundant eosinophilic cytoplasm, resembling rhabdomyoblasts (Fig. 2C). These tumor cells were discriminated from entrapped, atrophic muscle fibers by their cell atypia and continuity to adjacent tumor cells. Many of the tumor cells had vacuolated cytoplasm, and some showed a spider web-like appearance (Fig. 2D). These vacuoles were negative for both PAS and Oil red-O staining. Also, at the

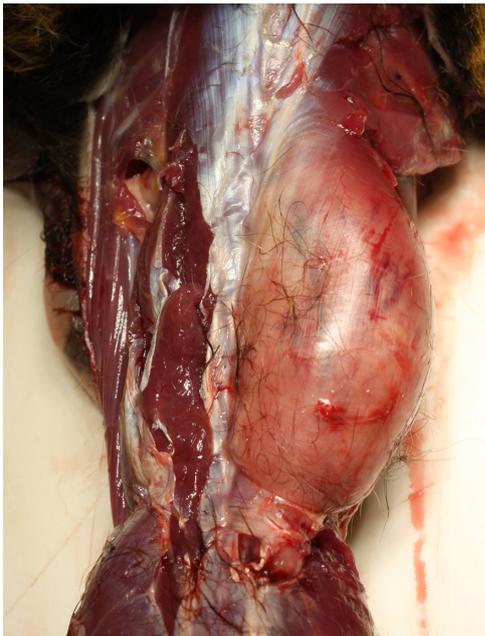


Fig. 1. Macroscopic appearance of the mass. A 2×2×5-cm intramuscular mass can be observed in the lower right back.

periphery of the mass (Fig. 2E), nests of round, clear tumor cells separated by thin fibrous septa were seen (Fig. 2F). Tumor cells invaded the spinal canal compressing the spinal cord (Fig. 2G), and metastasis to the lung was observed (Fig. 2H). In contrast to the primary mass, the metastatic lesion in the lung was almost exclusively composed of spindle-shaped tumor cells.

The results of the immunohistochemistry were similar between spindle-shaped tumor cells and round, clear tumor cells. Most tumor cells were positive for myogenin (Fig. 3A), desmin (Fig. 3B) and vimentin (Fig. 3C). Also, many of the tumor cells were positive for alpha-smooth muscle actin (α SMA) (Fig. 3D), while they were totally negative for myoglobin and cytokeratin.

Ultrastructurally, the tumor cells were characterized by distorted nuclei and distinct nucleoli (Fig. 4A). In the cytoplasm, the tumor cells contained bundles of myofilaments with Z-band-like structures (Fig. 4B). Also, some tumor cells had vacuoles in the cytoplasm (Fig. 4C). These vacuoles were filled with electron-lucent, flocculent materials, and a limiting membrane was not observed.

Thus, the tumor was diagnosed as a rhabdomyosarcoma based on the above-mentioned characteristics: rhabdomyoblasts in the tumor, positive reactions for myogenin, a specific marker of rhabdomyoblastic differentiation¹⁰, and myofilaments with Z-band-like structures in the cytoplasm. In this case, the tumor cells were negative for myoglobin, another specific marker of rhabdomyosarcoma. However, it is known that myoglobin expression can be seen only in well-differentiated tumors, while undifferentiated tumors are strongly positive for vimentin and myogenin, as seen in our case^{11,12}.

The differential diagnosis included other soft tissue sarcomas that could show partial rhabdomyoblastic differentiation, such as liposarcoma¹³, leiomyosarcoma¹⁴ and malignant peripheral nerve sheath tumor (malignant triton tumor)¹⁵. These tumors were excluded because in this case, the tumor cells uniformly showed rhabdomyoblastic characteristics and there was no evidence of differentiation into other tissues. Though many of the tumor cells were positive for α SMA, a widely used marker of smooth muscle cells, it has been reported that some rhabdomyosarcomas can also express this protein^{11,16}, and leiomyosarcomas are generally negative for myogenin.

Rhabdomyosarcoma is a malignant tumor derived from striated muscle cells or pluripotential mesenchymal

Table 1. Antibodies Used in This Study

Antibody	Clone	Dilution	Antigen retrieval ^a	Source ^b
Alpha-smooth muscle actin	1A4	1:100	Heat	Dako*
Cytokeratin	AE1/AE3	Prediluted	Heat	Dako**
Desmin	D33	Prediluted	Heat	Dako**
Myoglobin	Polyclonal	1:2000	–	Dako*
Myogenin	F5D	1:50	Heat	Dako**
Vimentin	V9	Prediluted	Heat	Dako**

^a Heat: pressure cooker/citrate buffer (pH 6.0), 10 minutes. –: Not conducted. ^b Dako*: Glostrup, Denmark. Dako**: Dako, Carpinteria, CA, U.S.A.

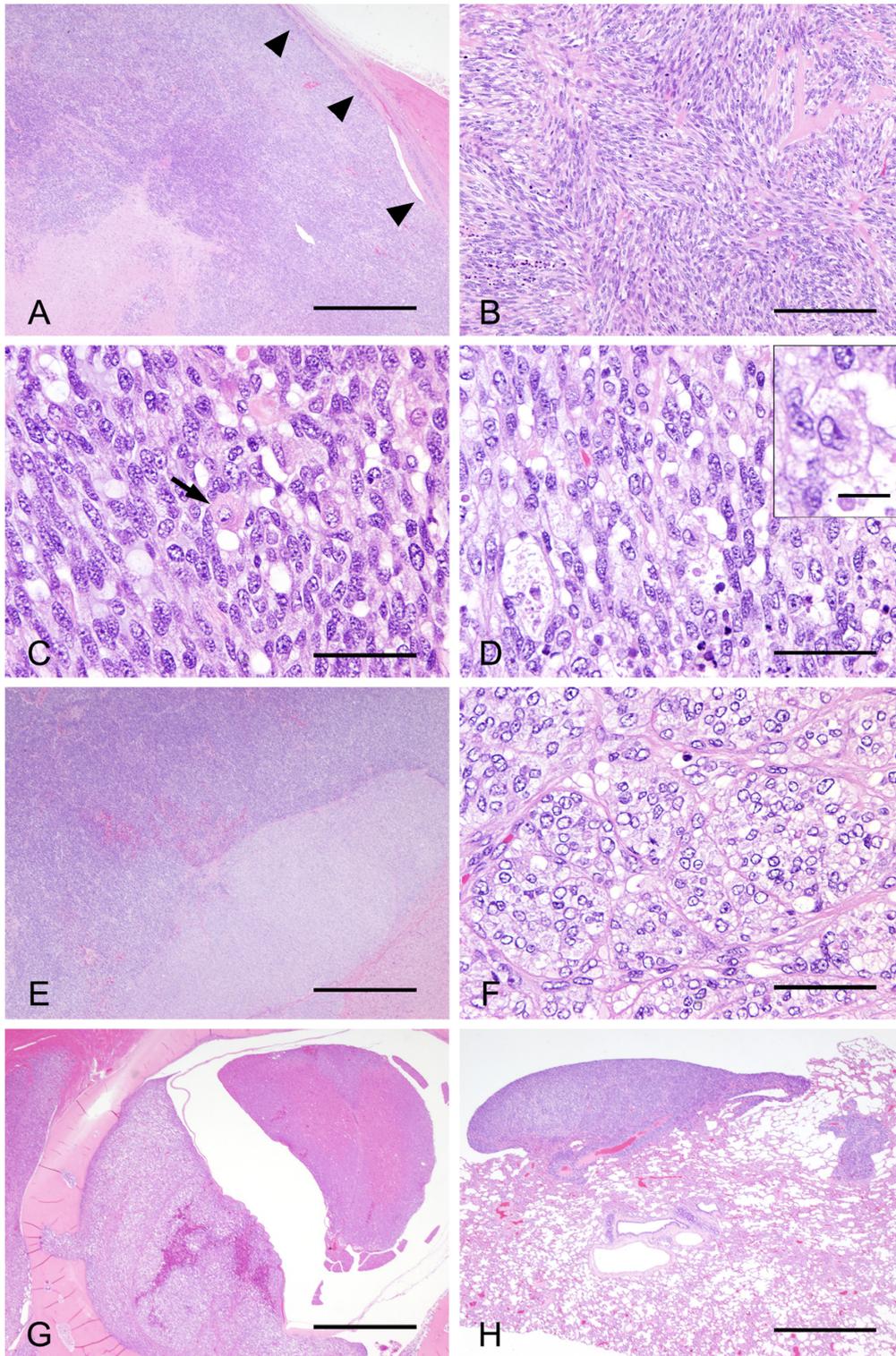


Fig. 2. Histological appearance of the mass. (A) The mass is surrounded by the fascia (arrowheads) with atrophic muscle fibers, and the center of the mass is necrotic. HE. Bar = 2 mm. (B) The mass is mainly composed of interlacing bundles of spindle-shaped tumor cells. HE. Bar = 400 μ m. (C) Tumor cells have elongated, blunt-ended nuclei and weakly basophilic, vaguely outlined cytoplasm. Some tumor cells have abundant eosinophilic cytoplasm, resembling rhabdomyoblasts (arrow). HE. Bar = 100 μ m. (D) Many of the tumor cells have vacuolated cytoplasm, and some show a spider web-like appearance. HE. Bar = 100 μ m. Inset: Higher magnification. Bar = 25 μ m. (E) At the periphery of the mass, a focus of clear cells can be seen. (F) Higher magnification of Fig. E. Nests of round, clear tumor cells separated by thin fibrous septa can be seen. HE. Bars = 2 mm (E) and 100 μ m (F). (G) Tumor cells invade the spinal canal and compress the spinal cord. HE. Bar = 2 mm. (H) Metastasis to the lung can be seen. HE. Bar = 2 mm.

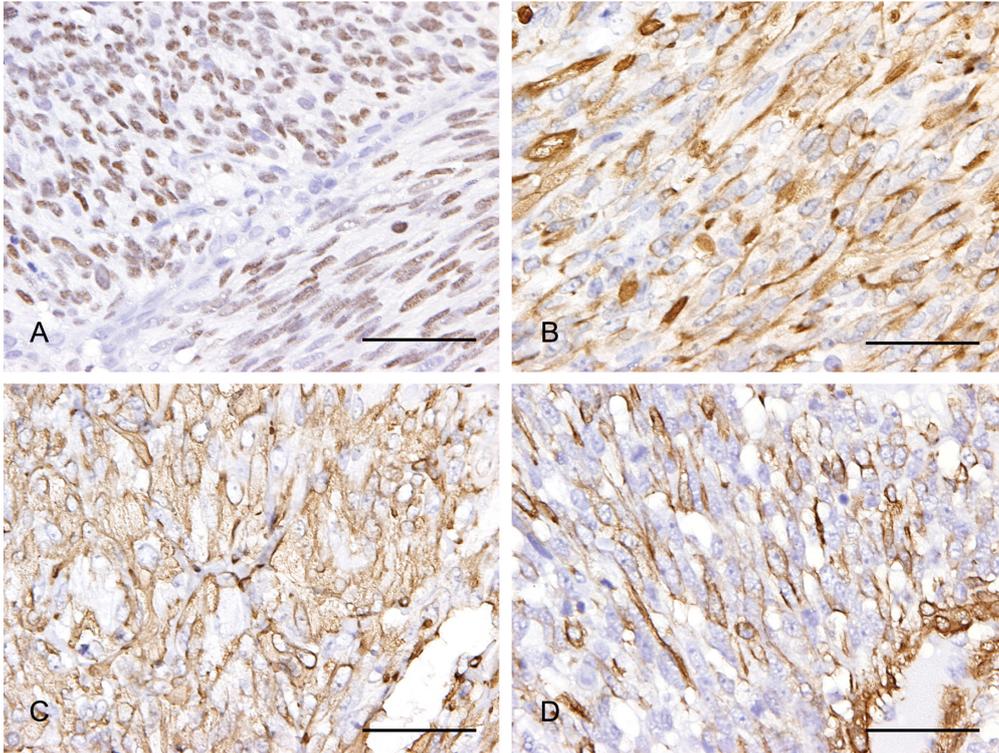


Fig. 3. Immunohistochemical findings of the mass. Most tumor cells are positive for myogenin (A), desmin (B), and vimentin (C). Also, many of the tumor cells are positive for alpha-smooth muscle actin (D). Bars= 100 μ m.

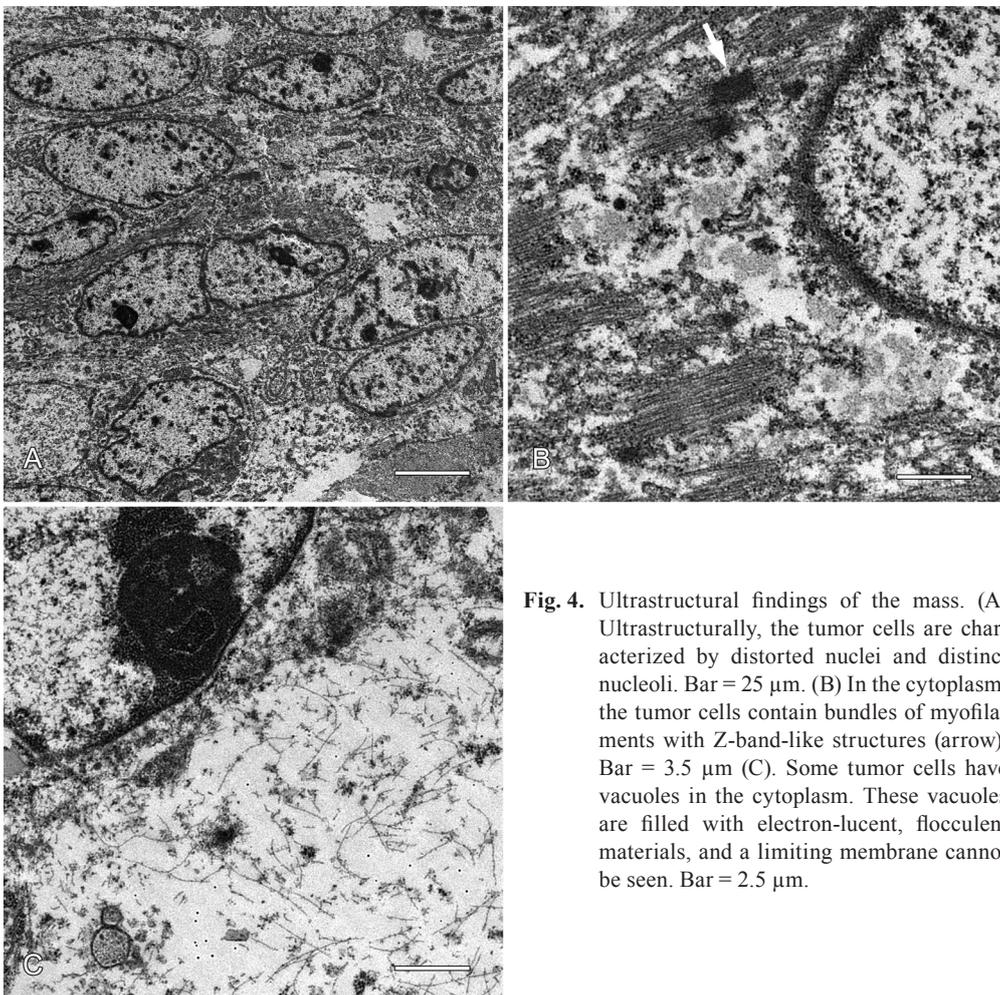


Fig. 4. Ultrastructural findings of the mass. (A) Ultrastructurally, the tumor cells are characterized by distorted nuclei and distinct nucleoli. Bar = 25 μ m. (B) In the cytoplasm, the tumor cells contain bundles of myofilaments with Z-band-like structures (arrow). Bar = 3.5 μ m (C). Some tumor cells have vacuoles in the cytoplasm. These vacuoles are filled with electron-lucent, flocculent materials, and a limiting membrane cannot be seen. Bar = 2.5 μ m.

stem cells¹¹. In our case, the mass was surrounded by the fascia with atrophic muscle fiber, suggesting an intramuscular origin of this tumor. Also, many of the tumor cells were vacuolated in the cytoplasm. It is known that some rhabdomyosarcomas accumulate lipids or glycogen and show clear cell morphology^{12,17}. However, we could not clarify the nature of those vacuoles, because they were negative for both PAS and Oil red-O staining, and ultrastructurally they were observed as vacuoles without limiting membranes.

In both humans and domestic animals, rhabdomyosarcomas are histologically classified into 3 types: embryonal, alveolar, or pleomorphic^{11,18}. In this case, the mass was composed of two populations—bundles of spindle cells resembling embryonal-type rhabdomyosarcoma, especially the spindle cell type^{18,19}, and nests of clear, round cells separated by thin fibrous septa resembling the alveolar type—and was most consistent with the mixed embryonal/alveolar type.

In the common marmoset, rhabdomyosarcoma has been induced by inoculation of Rous sarcoma virus²⁰. Also, McIntosh *et al.* reported a spontaneous nasopharyngeal tumor that was suspected as rhabdomyosarcoma, though the diagnosis was not confirmed²¹. To our knowledge, however, there has been no report of spontaneous rhabdomyosarcoma that was definitely diagnosed in this species.

In this report, we described the histological, immunohistochemical and ultrastructural characteristics of a spontaneous rhabdomyosarcoma in a common marmoset.

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