**Subcutaneous rhabdomyosarcoma in an old rabbit**

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**ABSTRACT.** An 11-year-old castrated male rabbit presented with a subcutaneous mass in the right hind limb. The mass comprised solid and round cells with eccentrically located nuclei, whereas the myxoid areas were composed predominantly of elongated fusiform cells with hyperchromatic nuclei embedded in Alcian Blue-positive myxoid stroma. Immunohistochemically, tumor cells in both areas were positive for desmin and vimentin. Ultrastructurally, the tumor cells in the solid areas had abundant myofilaments with electron dense Z-band structures. Based on these pathological findings, this case was diagnosed as rhabdomyosarcoma in a rabbit.

**KEYWORDS:** rabbit, rhabdomyosarcoma, subcutis

Rhabdomyosarcomas are skeletal muscle tumors that arise from pluripotent mesenchymal stem cells or striated myoblasts [5]. In domestic animals, rhabdomyosarcomas are rare neoplasms that account for less than 1% of all tumors. They are classified into the embryonal, alveolar and pleomorphic subtypes [3]. In rabbits, to the best of our knowledge, only one case of rhabdomyosarcoma has been reported [10]. Here, we add the case of rhabdomyosarcoma with various histopathological appearances, and the differential diagnosis of this unusual tumor is also discussed.

An 11-year-old castrated male cross-breed pet rabbit was presented to the local veterinary hospital with a rapidly growing subcutaneous mass in the crural regions of the right hind limb that had been present for 2 months. The surface of this unusual tumor is also discussed.

Microscopically, at low-power magnification, the mass was fairly well-demarcated and partially encapsulated by thin fibrous tissue. The tumor was divided into both solid and loosely packed myxoid areas (Supplemental Fig. S1b). Multiple small regions of coagulative necrosis were scattered throughout the solid areas. In the solid areas, the tumor cells showed hypercellularity and were composed predominantly of well-differentiated spindle-shaped to elongated forms arranged in an interlacing and storiform growth pattern (Fig. 2A). The tumor cells had indistinct cell borders, with oval to elongated hyperchromatic nuclei and single prominent nucleoli and a deeply eosinophilic fibrillary cytoplasm. Occasionally, elongated pleomorphic to multinucleated cells (Fig. 2B), strap-like cells and cross-striations (Supplemental Fig. S1c) in the cytoplasm were observed. Some tumor cells were large and round, with abundant eosinophilic cytoplasm and eccentrically located nuclei (Fig. 3). Anisocytosis and anisokaryosis were marked, and many mitotic figures (6–8 per high-power field, ×400) were noted.

NOTE

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An 11-year-old castrated male cross-breed pet rabbit was presented to the local veterinary hospital with a rapidly growing subcutaneous mass in the crural regions of the right hind limb that had been present for 2 months. The mass was excised and fixed in 10% neutral-buffered formalin solution. The mass measured 4.7 × 3.2 × 2.5 cm, and the cut surface had a varied appearance, including greyish solid areas (approximately 60%) and whitish myxoid areas with mild cystic changes (Fig. 1).

The mass was embedded in paraffin wax and cut into 3 µm thick sections. The sections were stained with hematoxylin and eosin (HE), Alcian Blue (pH 2.5) and phosphotungstic acid-hematoxylin (PTAH). Immunohistochemistry was performed as described previously [1] using the following primary antibodies: desmin (Dako-Japan, Kyoto, Japan), vimentin (Nichirei Biosciences, Tokyo, Japan), α-smooth muscle actin (α-SMA, Daco-Japan), cytokeratin (AE1/AE3, Daco-Japan), adipophilin (Progen Biotechnik, Heidelberg, Germany) and S-100 protein (Nichirei Biosciences). The antigen-antibody complexes were visualized using 3,3′-diaminobenzidine solution (Daco-Japan), and the sections were counterstained with hematoxylin. For electron microscopic examination, the formalin-fixed specimen was cut into 1-mm blocks, fixed in 1% buffered osmium tetroxide and embedded in epoxy resin. Sections, approximately 70 nm in thickness, were stained with uranyl acetate and lead citrate, and examined using a transmission electron microscope (H-7650, Hitachi, Tokyo, Japan).

Microscopically, at low-power magnification, the mass was fairly well-demarcated and partially encapsulated by thin fibrous tissue. The tumor was divided into both solid and loosely packed myxoid areas (Supplemental Fig. S1b). Multiple small regions of coagulative necrosis were scattered throughout the solid areas. In the solid areas, the tumor cells showed hypercellularity and were composed predominantly of well-differentiated spindle-shaped to elongated forms arranged in an interlacing and storiform growth pattern (Fig. 2A). The tumor cells had indistinct cell borders, with oval to elongated hyperchromatic nuclei and single prominent nucleoli and a deeply eosinophilic fibrillary cytoplasm. Occasionally, elongated pleomorphic to multinucleated cells (Fig. 2B), strap-like cells and cross-striations (Supplemental Fig. S1c) in the cytoplasm were observed. Some tumor cells were large and round, with abundant eosinophilic cytoplasm and eccentrically located nuclei (Fig. 3). Anisocytosis and anisokaryosis were marked, and many mitotic figures (6–8 per high-power field, ×400) were noted.
Within the myxoid areas, the tumor was composed mainly of elongated fusiform cells with hyperchromatic nuclei and a scant cytoplasm. They were loosely arranged in wavy lamellae and an occasional fascicular pattern in an abundant myxoid stroma (Supplemental Fig. S1d). Bizarre and multinucleated cells with a multivacuolated cytoplasm (spider web-like cells) were occasionally found (Fig. 4). Mitotic figures within the myxoid areas were infrequent (0–1 per high-power field, ×400). Intracytoplasmic vacuoles of tumor cells and myxoid stroma showed a strong positive signal for Alcian Blue (Fig. 4, inset; Supplemental Fig. S2a). PTAP staining demonstrated irregular cytoplasmic cross-theriati ons in many of the strap-like cells in solid areas (Supplemental Fig. S2b), but none were found in the myxoid areas.

Immunohistochemically, most of the tumor cells in both areas were strongly and diffusely positive for vimentin. On the other hand, desmin was intensely positive in solid areas (Supplemental Fig. S2c), but was weakly and sparsely positive in myxoid areas (Supplemental Fig. S2d). Bizarre and multinucleated cells with a multivacuolated cytoplasm in the myxoid areas were negative for desmin. Some tumor cells within the solid areas showed focally positive staining for α-SMA, but the tumor cells in the myxoid areas were negative. All tumor cells in both areas were negative for AE1/AE3, adipophilin and S-100.

Ultrastructurally, spindle to elongated tumor cells in solid areas generally had large and irregular-shaped nuclei, a markedly invaginated nuclear membrane with marginal heterochromatin and numerous cytoplasmic myofilaments. The myofilaments were haphazardly arranged, with a variable number of electron-dense rod-like patches appearing as Z-band structures, as well as free polyribosomes closely associated with them (myosin-ribosome complexes) (Fig. 5). The intracytoplasmic organelles, such as mitochondria, glycogen granules, rough endoplasmic reticulum and Golgi apparatus, were interspersed. In myxoid areas, elongated fusiform tumor cells and spider web-like cells had similar intracytoplasmic organelles, but bundles of myofilaments and Z-band structures as solid areas were not found. In spider web-like cells, intracytoplasmic organelles were mainly located at the perinuclear portions, and having electron lucent various sized vacuoles (200 nm to 4 μm in size) was observed throughout the cytoplasm (Fig. 6A and 6B).

Based on the above pathological findings, the present case was diagnosed as rhabdomyosarcoma. In the present case, the most characteristic histopathological findings were mixed proliferation of pleomorphic and embryonal components within the same tumor. These proliferative patterns are partly similar to previously reported embryonal rhabdomyosarcoma in domestic animals [3]. In the present study, spider web-like cells with multivacuoles in the cytoplasm are one of the characteristic findings in myxoid areas. Ultrastructurally, these tumor cells had abundant free ribosome, rough-surfaced endoplasmic reticulum and myofilaments. Therefore, we concluded that spider web-like cells are poorly or undifferentiated tumor component and originated from myoblasts. In human rhabdomyosarcoma and developing skeletal muscle, the expression patterns of myogenic protein depend on the degree of morphological differentiation [2]. In general, poorly differentiated myoblasts positively stained for vimentin, but other myogenic makers including desmin are negative. On the other hand, desmin is strong positive in well differentiated rhabdomyosarcoma. In the present case, most of the tumor cells in both areas were diffusely positive for vimentin, whereas desmin was sparsely positive in myxoid areas. These findings suggested that tumor cells in myxoid areas were poorly and/or undifferentiated tumor cells than those in solid areas.

In the present study, the differential diagnosis included myxoid liposarcoma, myxosarcoma, myxoid leiomyosarcoma and malignant schwannoma, because these tumors also have a myxoid matrix in the background. The diagnosis of myxoid liposarcoma is based primarily on histologic criteria of the mucopolysaccharide ground substance within the tumor, with tumor cells arranged in loose sheets within the myxoid stroma [5]. Additionally, the lipid vacuoles in the tumor cell cytoplasm are usually positive for vimentin, S-100 and adipophilin [11]. In this case, the tumor cells were negative for S-100 and adipophilin; therefore, the possibility of a myxoid liposarcoma could be ruled out. A myxosarcoma is a tumor arising from proliferating fibroblasts or multipotent mesenchymal cells and is generally characterized by the proliferation of spindle cells embedded in an abundant myxoid matrix. The tumor cells show restricted reactivities of desmin, S-100 and α-SMA antibodies [6]. Regarding its ultrastructural characteristics, a myxosarcoma has abundant

Fig. 1. The cut surface of the mass shows greyish solid (left) and whitish myxoid (right) areas with small cystic changes. Bar=1 cm.

Fig. 2. In the solid areas, the tumor cells show hypercellularity and arranges in an interfacing and storiform growth pattern (A). The tumor cells have oval to elongated hyperchromatic nuclei, and some of cells shows pleomorphism and multinucleated cells are intermixed (B, arrows). Hematoxylin and eosin stain. Bar=250 μm (A) and 50 μm (B).

Fig. 3. In the solid areas, large, round tumor cells with an abundant eosinophilic cytoplasm and eccentrically located nuclei (arrows) are observed. Hematoxylin and eosin stain. Bar=25 μm.

Fig. 4. In myxoid areas, multinucleated cells (arrows) with a multivacuolated cytoplasm (spider web-like cells) in the background of the myxoid stroma are seen. Hematoxylin and eosin stain. Bar=50 μm. Intracytoplasmic vacuoles of the multinucleated cells are positive (inset) for Alcian Blue. Bar=125 μm.

Fig. 5. In the solid areas, various sized myofilaments and electron-dense Z-band-like structures (arrows) are seen. N=nucleus. Electron microscopy. Bar=1 μm.

Fig. 6. Low magnification of spider web-like cells in myxoid areas (6A). Abundant cytoplasmic free ribosome, rough-surfaced endoplasmic reticulum and various sized vacuoles (asterisks) are observed. High magnification of spider web-like cells (6B). Myofilament-like structures are observed in the cytoplasm. N=nucleus. Electron microscopy. Bar=2 μm (6A), 100 nm (6B).
rough endoplasmic reticulum and numerous intracellular collagen fibers, but no cytoplasmic myofilaments and Z-band structures [9]. A myxoid leiomyosarcoma is an uncommon tumor where tumor cells are diffusely positive for α-SMA [7]. In our case, α-SMA staining was focally positive, and the characteristic electron microscopic findings (microfilaments, dense body, pinocytotic vesicles and basal lamina) observed in leiomyosarcomas were not evident [4]. A malignant schwannoma arises from Schwann cells and/or perineurial fibroblasts [8]. In rabbits, malignant schwannomas are characterized by the proliferation of wavy spindle-shaped cells arranged in short interwoven fascicles and perivascular whorls [10]. These tumors are positive for S-100, and basal lamina, and desmosome-like structures and cytoplasmic processes are found on ultrastructural examination [1, 8]. In our case, all of the tumor cells were negative for S-100, and the ultrastructural appearance of tumor cells was different from that of Schwann cells.

In rabbits, the most common cutaneous mesenchymal tumors are lipoma, myxosarcoma, malignant schwannoma, fibrosarcoma and leiomyosarcoma. These tumors frequently occur in intact young males with a mean age of 5–6 years [10], suggesting that sex hormones may directly influence tumor development. To the best of our knowledge, only one case of rhabdomyosarcoma in a rabbit has been reported [10], and the rabbit developed metastasis to another skin site within 1 month after surgery. The case presented herein occurred in an aged male rabbit that died as a result of pulmonary edema of unknown cause 96 days after surgery. After surgical excision, recurrence and metastatic lesions were not observed upon clinical examination and abdominal ultrasound examinations.

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