Case Report

Spontaneous Extraskeletal Osteosarcoma in a Rabbit (*Oryctolagus cuniculus*): Histopathological and Immunohistochemical Findings

Kavindra Kumara Wijesundera, Takeshi Izawa, Daisuke Fujita, Yuki Denda, Eiko Seto, Hiroshi Sasai, Mitsuru Kuwamura, and Jyoji Yamate*

1 Laboratory of Veterinary Pathology, Division of Veterinary Sciences, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 1-58 Rinku-oraikita, Izumisano City, Osaka 598-8532, Japan
2 Kitasuma Animal Hospital, 9-5-8 Yokoo, Suma-ku, Kobe City, Hyogo 654-0131, Japan

Abstract: A spontaneously occurring subcutaneous mass in the left forelimb of a nine-year-old rabbit (*Oryctolagus cuniculus*) was examined histopathologically and immunohistochemically. Clinically, edema and hemorrhage were seen around the mass. No connection of the tumor mass to the appendicular skeleton was found. The tumor was arranged in a solid growth pattern and irregular bundles, and neoplastic cells were polygonal to spindle-shape. Osteoid (positive for osteocalcin) and multinucleated giant cells were diffusely or focally seen. Neoplastic cells were positive for vimentin, osterix and Ki-67, indicating the nature of osteoblasts with proliferating activity, but negative for α-smooth muscle actin, desmin or CD204. Based on these findings, a diagnosis of extraskeletal osteosarcoma was made, a very rare tumor both in laboratory and pet rabbits. (DOI: 10.1293/tox.26.309; J Toxicol Pathol 2013; 26: 309–312)

Key words: rabbit, spontaneous tumor, extraskeletal osteosarcoma, immunohistochemistry

Osteosarcoma is the primary malignant tumor derived from bone tissue; therefore, the tumor is characterized by osteoid and immature bone formed by neoplastic osteoblasts. Osteosarcomas vary greatly in the amount and quality of matrix and in histological patterns. Canine osteosarcoma accounts for 85–98% of all canine bone tumors. In humans, osteosarcoma is the most common primary solid bone tumor in childhood and adolescence. Extraskeletal osteosarcoma (ESOS) is a rare malignant mesenchymal neoplasm, which is never attached to the skeleton, although the histopathological characteristics such as osteoid and bone formation are similar to those of bone-derived osteosarcomas. ESOS has been reported in dogs, cats, hamsters, a rat, a hedgehog, a maned wolf and a goat as an infrequent tumor. In aged rabbits, uterine adenocarcinoma is the most common spontaneous tumor followed by lymphomas. A few cases of spontaneously occurring osteosarcomas have been reported in rabbits, out of them, to our knowledge, only one case was diagnosed as an ESOS arising in the upper lip. Because of the rarity of this tumor type and its necessity for pet and laboratory animals, we herein report the detailed histopathological and immunohistochemical characteristic of a case of rabbit ESOS.

A nine-year-old male rabbit (*Oryctolagus cuniculus*) (1.8 kg body weight, mixed, colored) was brought to a private animal hospital with a complaint of hemorrhage and edema around the dewclaw of the left forelimb. A subcutaneous solid mass, 3.5 cm × 1.5 cm × 1.5 cm, was surgically removed. The cut surface of the formalin-fixed mass appeared grayish white and pink color. Two months after the surgical resection, the rabbit was re-admitted with an ulcerative mass at the resection site. The recurrent mass resembled the primary mass in terms of gross morphology.

The subcutaneous masses were fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned at 3–5 μm. Besides hematoxylin and eosin (HE) staining, the Periodic acid-Schiff (PAS), von Kossa and azan-Mallory methods were performed for histopathology. Immunohistochemical labeling was performed with peroxidase conjugated secondary antibody (Histofine Simple Stain MAX PO; Nichirei Inc., Tokyo, Japan). Primary antibodies used were osteocalcin (clone, OC4-30; 1:500; GeneTex, CA, USA), vimentin (clone, V9; 1:500; Dako, Denmark), desmin (clone, D33; 1:200; Dako, Denmark), CD204 (clone, SRA-E5; 1:200; TransGenic, Japan), Ki-67 (clone, MIB-1; 1:200; Dako, Denmark), α-smooth muscle actin (α-SMA) (clone, 1A4; 1:1000; Dako, Denmark), S-100 (1:200; Abcam, UK), and osterix (1:200; Abcam, UK). Positive reac-

Received: 21 March 2013, Accepted: 4 April 2013

*Corresponding author: J Yamate (e-mail: yamate@vet.osakafu-u.ac.jp)

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tions were visualized with 3,3′-diaminobenzidine (DAB; Vector Laboratories, Inc., Burlingame, CA, USA). Sections were lightly counterstained with hematoxylin. For negative controls, tissue sections were treated with mouse or rabbit nonimmune serum instead of the primary antibody. Some normal rabbit tissues were used as positive controls.

On histopathological examination, the tumor was composed of solid growth or irregular bundles of polygonal to spindle-shaped neoplastic cells including areas of osteoid and osseous formation (Fig. 1B). Tumor cells had oval to elongated hyperchromatic nuclei with marked pleomorphism. Mitotic figures were frequently present. Multinucleated giant cells were focally or sporadically observed (Fig. 1B; inset). The osseous matrix was stained black and blue by the von Kossa stain and, indicating the presence of calcium salt. Bar = 200 µm. (D) Osseous matrix is stained blue by the azan-Mallory stain. Bar = 200 µm. (E) Cytoplasm of neoplastic cells (arrows) and multinucleated giant cells is positive for vimentin. Immunohistochemistry, counterstained with hematoxylin. Bar = 200 µm. (F) Nuclei of many neoplastic cells (arrow) show strong positivity for Ki-67. Immunohistochemistry, counterstained with hematoxylin. Bar = 200 µm. (G) The osseous matrix (arrows) reacts positively with the anti-osteocalcin antibody. Immunohistochemistry, counterstained with hematoxylin. Bar = 200 µm. (H) Nuclei of neoplastic cells (arrows), particularly within osseous tissues, show a positive reaction to osteocalcin, indicative of the nature of osteoblasts. Immunohistochemistry, counterstained with hematoxylin. Bar = 200 µm.

In conclusion, because of the rarity in occurrence, a spontaneous ESOS, which was encountered in an aged pet rabbit, was characterized by histopathological and immunohistochemical methods. The findings could be useful for differential diagnosis of mesenchymal tumors without connection to bone in laboratory animals.
Acknowledgment: The authors would like to thank Dr. Mika Nagaika of Ishihara Sangoyo Kaisha, Ltd., Japan, who kindly provided assistance in osterix immunohistochemistry.

References