

Case Report

Spontaneous extraskeletal osteosarcoma in the neck skeletal muscle of a Crlj:CD1 (ICR) mouse

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Abstract: Extraskeletal osteosarcoma is extremely rare in humans and animals, especially in rodents. This is the first case report on spontaneous extraskeletal osteosarcoma in the neck skeletal muscle of a Crlj:CD1 (ICR) mouse (36 weeks, dead). Necropsy revealed a solid white mass located in the neck skeletal muscle (scalenus muscle). Histological examination showed that the tumor consisted of atypical polygonal cells, a small osteoid clump, and bone tissue. Mitotic figures were observed. Serial sections showed that neoplastic cells lacked clear invasive proliferation to adjacent normal skeletal muscle and continuity with normal bone tissue. Immunohistochemical analysis showed that the neoplastic cells were positive for osteocalcin, osterix, vimentin, and S-100. Based on these results, the tumor was diagnosed as extraskeletal osteosarcoma in the neck skeletal muscle. (DOI: 10.1293/tox.2020-0083; J Toxicol Pathol 2021; 34: 147–150)

Key words: extraskeletal osteosarcoma, neck skeletal muscle, spontaneous tumor, mouse

Osteosarcoma is a malignant tumor that normally develops in the bone tissue; however, there have been few reports on extraskeletal osteosarcoma (EO) in rats and mice^{1–8}. Here, we describe the first reported case of spontaneous EO in the neck skeletal muscle of a mouse. The animal was a male Crlj:CD-1 (ICR) mouse (Charles River Laboratories Japan Inc., Kanagawa, Japan) and allocated to the intact group for the toxicological study. In that study, the animals were individually housed in stainless mesh cages; moreover, they were maintained at a temperature of $23 \pm 3^\circ\text{C}$ and relative humidity of $50 \pm 20\%$ with air ventilation at 17 times/h and 12 h of illumination (0700 h to 1900 h). The animals had free access to a pellet diet (irradiation-sterilized CRF-1 LID 30, Oriental Yeast Co., Ltd., Tokyo, Japan) and water. The experiment was conducted in compliance with the Act on Welfare and Management of Animals (Act No. 105, 1973), “Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain” (Notice No. 88 of the Ministry of Environment, 2006), and “Basic Guidelines for Animal Experimentation in the Research Laboratories under the Jurisdiction of the Ministry of Agriculture, Forestry and Fisheries” (18-Noukai-No.307, 2006).

The animal died at 36 weeks of age. Visual inspection did not show any abnormal changes. Necropsy revealed a

solid white mass ($5 \times 4 \times 4$ mm) in the neck skeletal muscle (scalenus muscle) (Fig. 1A). There was no continuity with the normal bone (spine) at necropsy and histological examination using serial sections (Fig. 1B and C). No other body mass was observed.

The observed mass and adjacent neck skeletal muscle were removed and fixed in 10% neutral-buffered formalin, embedded in paraffin wax, and sectioned at a thickness of approximately 4 μm . Subsequently, the sections were stained with hematoxylin and eosin (H&E). Other mass sections were subjected to immunohistochemistry and phosphotungstic acid hematoxylin (PTAH) staining. The primary antibodies used for immunohistochemistry were as follows: osteocalcin (polyclonal, diluted 1:200, Bioss Antibodies, Woburn, MA, USA), osterix (polyclonal, diluted 1:1500, Abcam, Cambridge, UK), cytokeratin AE1/AE3 (monoclonal, diluted 1:250, Abcam), vimentin (polyclonal, diluted 1:500, Abcam), S-100 (monoclonal, 1:2000, Abcam), α -smooth muscle actin (α -SMA) (monoclonal, 1:2000, Abcam), myogenin (monoclonal, 1:100, Abcam), and PCNA (polyclonal, 1:600; DakoCytomation, Osaka, Japan).

Histologically, the mass was located on the skeletal muscle surface. The neoplastic cells had atypical polygonal nuclei and scant-to-moderate polygonal eosinophilic cytoplasm with a solid growth pattern. Mitotic figures were observed. The proliferative region showed small clumps of osteoid-like eosinophilic matrix and highly differentiated bone tissue (Fig. 2A and B). The mass was not clearly encapsulated; however, there was no clear invasive proliferation of the adjacent skeletal muscle; moreover, lymphocyte infiltration was noted in the boundaries (Fig. 2C). No vessel invasion was observed.

Immunohistochemically, the neoplastic cells were posi-

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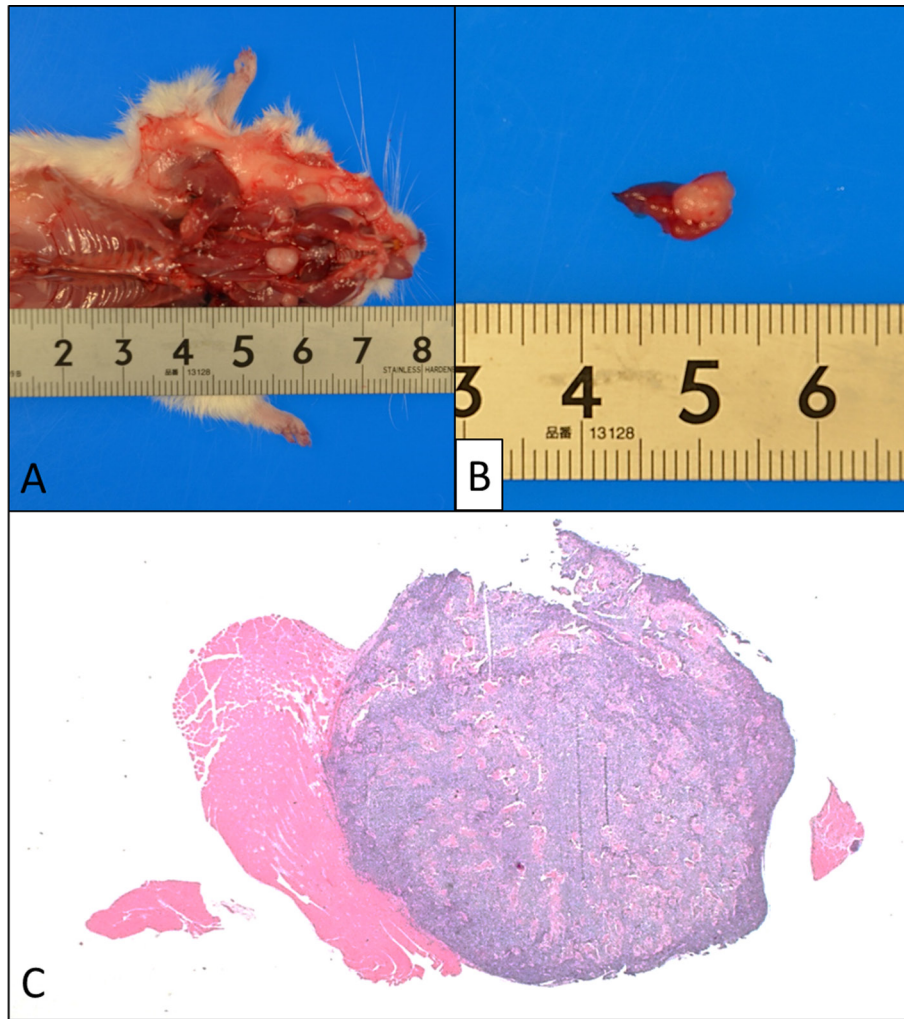


Fig. 1. Macroscopic findings. A, B. Solid white mass in the neck skeletal muscle (scalenus muscle) ($5 \times 4 \times 4$ mm). C. The mass showed continuity only to the neck skeletal muscle, with no mass observed in other organs.

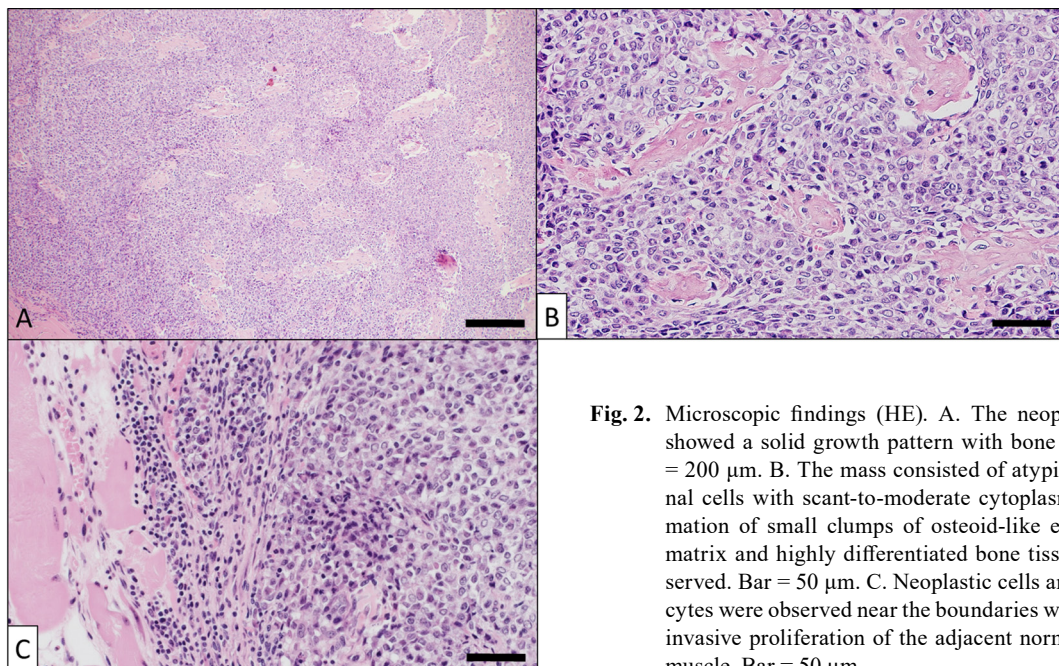


Fig. 2. Microscopic findings (HE). A. The neoplastic cells showed a solid growth pattern with bone tissue. Bar = $200 \mu\text{m}$. B. The mass consisted of atypical polygonal cells with scant-to-moderate cytoplasm, and formation of small clumps of osteoid-like eosinophilic matrix and highly differentiated bone tissue was observed. Bar = $50 \mu\text{m}$. C. Neoplastic cells and lymphocytes were observed near the boundaries without clear invasive proliferation of the adjacent normal skeletal muscle. Bar = $50 \mu\text{m}$.

tive for osteocalcin, osterix, vimentin, S-100, and PCNA but negative for cytokeratin AE1/AE3, α -SMA, and myogenin (Fig. 3A–E). PTAH staining showed no cross-striations in neoplastic cells (Fig. 3F).

H&E staining indicated that the tumor was an osteosarcoma, specifically EO. However, based on the affected organ and pleomorphic proliferative pattern, rhabdomyosarcoma was considered a differential diagnosis. Therefore, additional immunohistochemistry and PTAH staining were performed. Osteocalcin and osterix are reliable markers for osteosarcoma, while myogenin is a marker for rhabdomyosarcoma. Some EO cases have been shown to be positive for vimentin, S-100, and α -SMA^{2–7}. PCNA was used to determine proliferation activity. Moreover, PTAH staining was used to check for muscle fiber cross-striations in the neoplastic cells of rhabdomyosarcoma. Based on these results, the tumor was diagnosed as an EO in the skeletal muscle

of the neck. There are various osteosarcoma subtypes, including eburnating (osteoplastic), chondroblastic, osteoclastic, anaplastic, osteoblastic, fibroblastic, telangiectatic (vascular), and compound (mixed) types^{9–11}. In this study, the tumor was considered the osteoblastic type because it was composed of neoplastic osteoblasts and highly differentiated with varying amounts of osteoid and bone tissues. In conclusion, this report presents the first case of spontaneous EO in the neck skeletal muscle of a Crlj:CD1 (ICR) mouse and describes the morphological and immunohistochemical characteristics of the tumor.

Disclosure of Potential Conflicts of Interest: The authors declare that they have no conflicts of interest.

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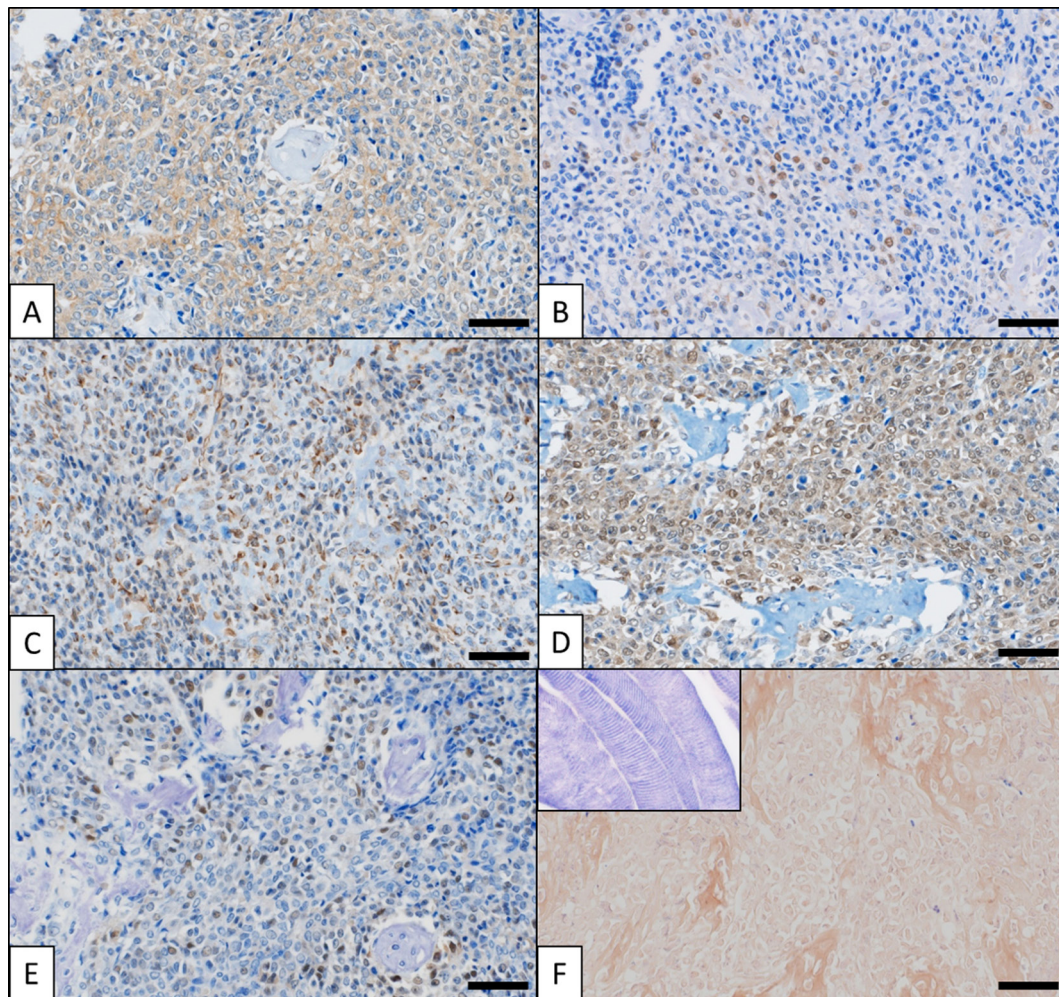


Fig. 3. Microscopic findings (immunohistochemistry and special staining). A. Most neoplastic cells were positive for osteocalcin (cytoplasm). Bar = 50 μ m. B. Some neoplastic cells were positive for osterix (nucleus). Bar = 50 μ m. C. Most neoplastic cells were positive for vimentin (cytoplasm). Bar = 50 μ m. D. Most neoplastic cells were positive for S-100 (nucleus and cytoplasm). Bar = 50 μ m. E. Some neoplastic cells were positive for PCNA (nucleus). Bar = 50 μ m. F. All neoplastic cells showed no muscle fiber cross-striations and were negative for PTAH (inner box: positive control). Bar = 50 μ m.

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