

Case Report

Spontaneous Fibrosarcoma with Pleomorphic Appearance in an Aged Brown Norway Rat

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Abstract: An 18-month-old male Brown Norway (BN) rat showed a grayish-white subcutaneous mass in the right cheek. Histologically, the mass was composed of highly pleomorphic cells producing collagen. Immunohistochemical analysis showed that the tumor cells were strongly positive for vimentin and partially positive for Ki-67; however, they were negative for ED-1, ED-2, S-100, cytokeratin, desmin and myoglobin. Ultrastructurally, the cytoplasm of the tumor cells contained well-developed rough endoplasmic reticulum. Thus, the tumor had no characteristic feature other than collagen production and was diagnosed as a fibrosarcoma. (*J Toxicol Pathol* 2010; 23: 261–263)

Key words: fibrosarcoma, Brown Norway rat

Introduction

Fibrosarcomas, originating from pluripotential mesenchymal stem cells or fibroblasts, are common primary mesenchymal tumors in rats¹. Generally, the tumors are composed of interlacing bundles of monomorphic, fusiform spindle cells producing various amounts of collagen². However, more anaplastic tumors can have marked cellular pleomorphism³, making diagnosis more difficult. In this study, we report a fibrosarcoma characterized by a highly pleomorphic appearance that occurred in the subcutis of an aged BN rat.

The BN rat was purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan) and kept without treatment for its entire life span. At 18 months of age, the animal showed swelling of the right cheek with a severe ulcer (Fig. 1) and was euthanized by exsanguination via the abdominal aorta under isoflurane anesthesia. The procedures for animal care and housing were in compliance with our institutional guidelines for the care and use of laboratory animals. At necropsy, a 1.5 × 1.5 × 0.5-cm subcutaneous grayish-white mass was observed in the right cheek. The mass was poorly demarcated and adherent to surrounding tissues. Tissue samples were taken from the mass, lungs, liver, kidneys, spleen, mandibular glands and mandibular lymph nodes and fixed in 10% neutral buffered formalin for histological examination.

The tissue samples were then dehydrated, embedded in paraffin wax, sectioned at 3 μm and stained with hematoxylin and eosin (H.E.). The sections of the mass were also stained by Masson's trichrome method. For immunohistochemical examination, the sections of the mass were subjected to a labeled polymer method using Histofine Simple Stain Rat MAX-PO (MULTI) (Nichirei Biosciences Inc., Tokyo, Japan) for antibodies against ED-1 (MCA341R; 1:400, AbD Serotec, Oxford, UK), ED-2 (MCA342R; 1:50, AbD Serotec), Ki-67 (M7248; 1:75, Dako, Carpinteria, CA, USA), S-100 (Z0311; 1:500, Dako, Carpinteria, Glostrup, Denmark), cytokeratin (N1590; predilution, Dako, Carpinteria, CA, USA), vimentin (N1521; predilution, Dako, Carpinteria, CA, USA), desmin (N1526; predilution, Dako, Carpinteria, CA, USA), and myoglobin (A0324; 1:1000, Dako, Carpinteria, Glostrup, Denmark) and were counterstained with hematoxylin. For electron microscopic examination, small pieces of the mass fixed in 10% neutral buffered formalin were refixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide and routinely embedded in Epon resin. Ultra-thin sections of the selected areas were prepared, contrasted with hafnium chloride and lead citrate and examined using a Hitachi 7600 transmission electron microscope (Hitachi High-Technologies Corporation, Tokyo, Japan).

Histologically, the mass was poorly delineated and non-capsulated (Fig. 2A) and was composed of highly pleomorphic cells with medium to abundant basophilic cytoplasm and round to oval nuclei (Fig. 2B). Multinucleated giant cells and mitotic figures were sparsely seen (Fig. 2C). The tumor cells were separated by significant amounts of eosinophilic matrix, which were stained blue by Masson's

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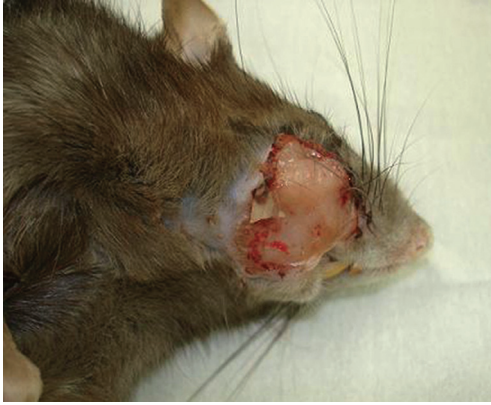


Fig. 1. Macroscopic appearance of the mass. The animal showed swelling of the right cheek with a severe ulcer.

trichrome staining (Fig. 2D), suggesting collagen production by the tumor cells. The results of the immunohistochemical analysis are summarized in Table 1. The tumor cells were strongly positive for vimentin (Fig. 2E) and partially (approximately 20%) positive for Ki-67 (Fig. 2F); however, they were completely negative for ED-1, ED-2, S-100, cytokeratin, desmin and myoglobin. Multinucleated giant cells showed the same reactions as other tumor cells for these markers, suggesting the common origin of these cells. Metastasis was not detected in other tissues histopathologically examined. Ultrastructurally, the tumor cells were surrounded by collagen fibers, that were about 50 nm in diameter. The nuclei of the tumor cells were irregularly shaped and had obvious nucleoli (Fig. 3A). The cytoplasm contained well-developed rough endoplasmic reticulum and a small number of mitochondria (Fig. 3B). Thus, the tumor

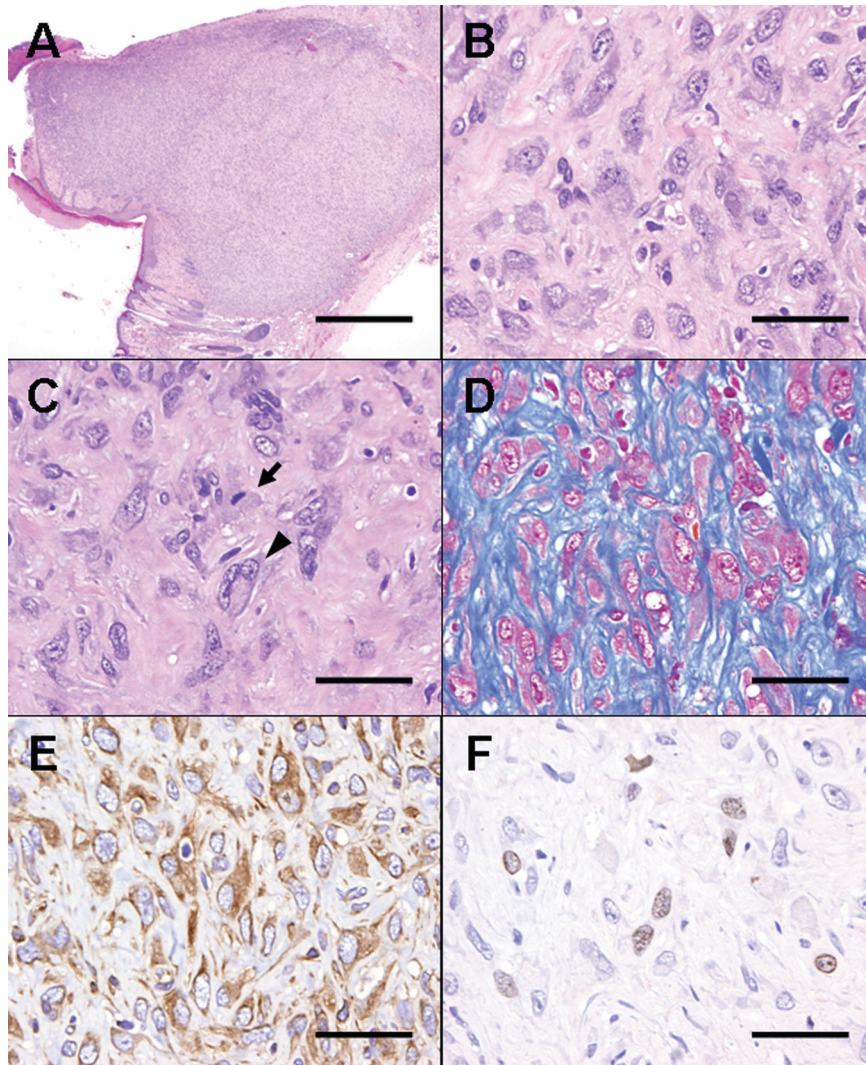


Fig. 2. Histological and immunohistochemical findings of the mass. The mass was poorly delineated and noncapsulated, and the overlying epidermis was ulcerated (A). Normal skin adjacent to the mass can be seen in the bottom of the picture. B and C show higher magnification of the central area of A. The mass was composed of highly pleomorphic cells (B). Multinucleated giant cells (arrowhead) and mitotic figures (arrow) were sparsely seen (C). H.E. stain. Bars=4 mm (A) and 200 μ m (B and C). The eosinophilic matrix among the tumor cells was stained blue by Masson's trichrome staining (D). Bar=200 μ m. The tumor cells were strongly positive for vimentin (E) and partially positive for Ki-67 (F). Bars=200 μ m.

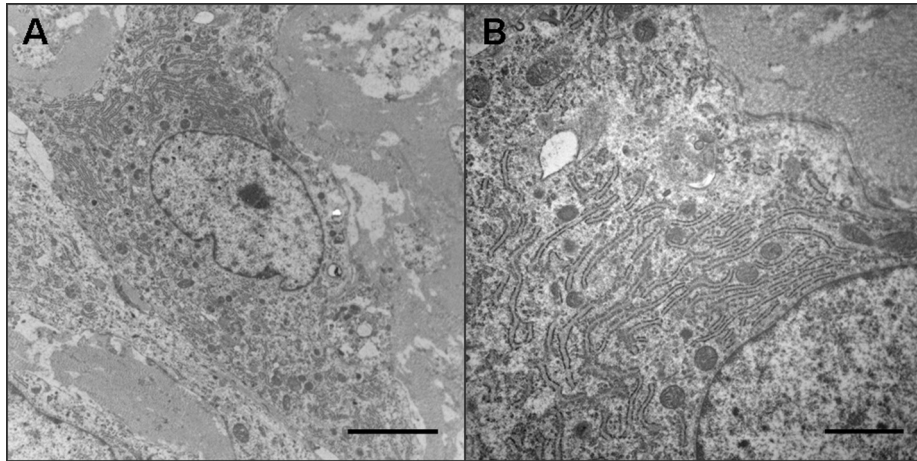


Fig. 3. Ultrastructural findings. Gross appearance of the tumor cells (A) and higher magnification (B). The tumor cells were surrounded by collagen fibers that were about 50 nm in diameter and had irregularly shaped nuclei with obvious nucleoli (A). The cytoplasm contained well-developed rough endoplasmic reticulum and small number of mitochondria (B). Bars=3 μ m (A) and 1.5 μ m (B)

Table 1. Immunohistochemistry Summary

Antibody	Reactivity
Vimentin	++
Ki-67	+
ED-1	–
ED-2	–
S-100	–
Cytokeratin	–
Desmin	–
Myoglobin	–

Levels of reactivity: ++, strongly positive; +, slightly positive; –, negative.

had no characteristic feature other than collagen production, and was diagnosed as a fibrosarcoma.

The differential diagnosis included rhabdomyosarcoma, malignant melanoma and malignant fibrous histiocytoma (MFH). Rhabdomyosarcomas, malignant tumors of striated muscles, are rare in rats. Histologically, the tumors are highly pleomorphic and composed of interlacing bundles of myoblast-like cells. Most tumors have positive reactivities for desmin and myoglobin, unlike the present case. Malignant melanomas are occasionally seen in the skins of aged BN rats⁴. The tumors are composed of pleomorphic cells with or without brown pigments in their cytoplasm. Most of the tumors show a positive reaction for S-100 and possess premelanosomes⁵. In the present case, the tumor cells were negative for S-100, and no premelanosomes were detected ultrastructurally. MFHs rarely occur spontaneously in rats. Histologically, MFH tumors are composed of storiform or cartwheel patterns of neoplastic fibroblasts interspersed with pleomorphic histiocytes that show positive reactions for ED-1 or ED-2⁶. MFH of the pleomorphic type is usually characterized by the appearance of multinucleated tumor giant cells, as seen in the present case. However, the negative reactions for ED-1 and ED-2 excluded histiocytic

differentiation of these tumor cells.

Though fibrosarcomas are generally characterized by fusiform spindle cells, more anaplastic tumors, which have a pleomorphic appearance, are infrequently encountered. In the latter cases, the diagnosis can be confusing, but fibrosarcomas are differentiated from other tumors by their collagen producing activity and negative reactions for various immunohistochemical markers for other mesenchymal tumors, except for vimentin.

In conclusion, the present case was diagnosed as a fibrosarcoma based on histological, immunohistochemical and ultrastructural findings. To our knowledge, fibrosarcoma in a BN rat has never been reported, and this suggests it was a rare case of fibrosarcoma occurring spontaneously in a BN rat.

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