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Case Report

A Case of Spontaneous Malignant Hibernoma in a Crl:CD(SD)IGS Rat

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Abstract: A firm, tan, well-circumscribed mass that measured 25 × 30 × 35 mm was observed in the thoracic cavity of a 53-week-old male Crl:CD(SD) IGS rat. Histologically, the mass was encapsulated by fibrous tissue and contained fibrovascular septae. Tumor cells were compactly arranged, and most were oval to polygonal in shape with multivacuolated cytoplasm and a centrally located nucleus. In some parts of the tumor, marked cellular atypia and frequent mitoses were evident. Vacuoles in cytoplasm were positive for oil red O. The tumor cells were characterized ultrastructurally by abundant, round to oval mitochondria with transverse closely-packed cristae. Tumor cells were immunohistochemically positive for uncoupling protein 1 (UCP-1). Several thrombi and hemorrhagic or necrotic foci were also observed within the tumor mass. Vascular invasion of the tumor capsule was observed; however, invasion of surrounding tissues or metastases were not observed. Based on the pathology findings, this case was diagnosed as a malignant hibernoma. (J Toxicol Pathol 2009; **22**: 205–208)

Key words: rat, spontaneous, malignant hibernoma, cellular atypia, vascular invasion, UCP-1

Hibernoma is a neoplasm originating from brown adipose tissue. There have been recent reports on drug-induced hibernomas in rats[1] and an unusually high incidence of spontaneous hibernomas in a carcinogenicity study in Wistar-Han rats[2]. In general, the incidence of spontaneous hibernoma in rats is low. In rats, hibernomas are most commonly seen in thoracic cavity as a space-occupying mass, which often relates to the cause of death or moribund condition after labored respiration or posterior paresis in the case of invasive growth into the vertebral column and spinal cord[2–4]. Benign hibernoma in rats is composed of well-differentiated tumor cells with no atypia, whereas cellular pleomorphism and increased mitotic activity are described as cytological and histological features of malignant hibernomas. Additionally, malignancy is based on the diagnostic features of local tissue invasion or distant metastasis[3,4].

We encountered a case of malignant hibernoma that occurred spontaneously in a rat, and the histopathological, ultrastructural and immunohistochemical features of the case are described in this report.

The animal was a 53-week-old male Crl:CD(SD) IGS rat that was part of a vehicle control group dosed with 1.5% carboxymethylcellulose in distilled water at a volume of 5 mL/kg in a 2-year carcinogenicity study. The animal was purchased from Charles River Laboratories (Raleigh, NC, USA) and housed in a suspended stainless-steel cage. Environmental control for the animal room was set to maintain a temperature of 18 to 26°C, a relative humidity of 30 to 70% and a 12-hour light/12-hour dark cycle. The animal was provided with certified rodent diet (Harlan Teklad) and water *ad libitum*. The animal was found dead during the 47th week of the study. Prior to dying, the animal had a three-week clinical course of labored respiration, decrease in food consumption and body weight loss. At necropsy, the thoracic cavity contained a firm, tan, well-circumscribed mass that measured 25 × 30 × 35 mm, partially adherent to the thoracic aorta, esophagus and lung. Labored respiration and decrease in food consumption observed before the animal died were probably due to the location and size of the mass.

The mass and other organs and tissues were fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin, sectioned at 3 μm and stained with hematoxylin and eosin (H&E). Additional sections from the mass were stained by Periodic Acid-Schiff (PAS) reaction, von Kossa's method, Schmorl method and with Berlin blue stain in accordance with standard procedures. Oil red O staining was performed on formalin-fixed, frozen, 12 to 14-μm-thick specimens. Immunohistochemical analyses were also conducted on paraffin-embedded sections. Primary

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antibodies used in this study were rabbit anti-human UCP-1 antibody (Abcam, Cambridge, UK, code No. ab10983, dilution 1:500) and mouse anti-rat CD68 antibody (ED1; Serotec, Oxford, UK, code No. MCA341R, dilution 1:500). Immunohistochemistry for UCP-1 was conducted as follows: after deparaffinization of the sections, antigen retrieval was performed in a pressure cooker with 0.01M citrate buffer (pH 6.0) for 10 minutes. The sections were treated with 0.03% H_2O_2 in methanol to quench the endogenous peroxidase activity, followed by the incubation with 1% bovine serum albumin to block nonspecific binding sites. The sections were then incubated with anti-UCP-1 antibody for 1 hour at room temperature. Visualization was performed by the universal immunoperoxidase polymer method (Histofine Simple Stain MAX-PO (R), Nichirei Bioscience, Tokyo, Japan). Immunohistochemistry for CD68 was conducted as follows: after deparaffinization of the sections, antigen retrieval was performed by 0.4 mg/mL Proteinase K in Tris buffered saline (pH 7.6) supplemented with 0.05% Tween 20 at room temperature for 10 minutes. The sections were treated with 3% H_2O_2 in distilled water to quench the endogenous peroxidase activity, followed by the incubation with 1% bovine serum albumin to block nonspecific binding sites. The sections were then incubated with ED1 for 2 hours at room temperature. Visualization was performed by the labeled-streptavidin-biotin method (LSAB 2 System-HPR, DakoCytomation, Glostrup, Denmark). For ultrastructural examination, small pieces of formalin-fixed tissue were transferred to 2.5% glutaraldehyde, post-fixed in 2% osmium tetroxide, dehydrated, embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined under a Hitachi H7600 transmission electron microscope.

Histologically, the mass was almost completely encapsulated by fibrous connective tissue. The tumor mass showed a lobular appearance with fibrovascular septa that extended from the capsule. The tumor cells were compactly arranged, and most of them were oval to polygonal in shape with multivacuolated cytoplasm and a centrally located nucleus. Vacuoles in tumor cells were positive for oil red O (Fig.1, inset). Those architectural and cytological characters revealed this tumor was of brown fat tissue origin. Some clusters of tumor cells with univacuolated cytoplasm and eccentric nuclei were also seen. Tumor cells in most part of the tumor showed little atypia and few mitoses. In other parts, on the other hand, areas comprised of atypical tumor cells exhibiting anisocytosis, higher nuclear-cytoplasmic ratio and marked nuclear pleomorphism including karyomegaly and multinucleation were present (Fig. 1). The cytoplasm of atypical tumor cells was eosinophilic with fewer and smaller vacuoles than non-atypical tumor cells. In these areas, frequent mitoses were also evident (Fig. 2). Vascular invasion with tumor tissue projection to the vascular cavity was observed in a portion of the tumor capsule (Fig. 3). Multiple, small to medium-sized necrotic foci with slight calcification, several thrombi that obstructed medium-sized blood vessels and occasional, mild hemorrhages were observed within the tumor mass. Metastasis or invasion toward the thoracic aorta, esophagus or lung or distant metastasis to other organs was not observed.

Ultrastructurally, tumor cells were characterized by abundant, round to oval mitochondria with transverse closely-packed cristae and intramatrical dense bodies. Various numbers of non-coalescing vacuoles were also observed in cytoplasm.

Immunohistochemically, the cytoplasm of the tumor cells was strongly positive for UCP-1 as well as normal brown adipocytes present around the thymus. Tumor cells were positive for UCP-1 even in the areas of cellular atypia (Fig. 4).

Groups of macrophages, immunohistochemically positive with ED1 antibody (data not shown), laden with yellow to brown pigments were occasionally observed within the fibrous septa or capsule. Those pigments were stained positively with Berlin blue stain or, with PAS and/or stained blue to green with Schmorl's stain, suggesting the presence of hemosiderin and lipofuscin. Clusters of macrophages with lipofuscin and hemosiderin pigments have been reported to be a feature in hibernoma of rats regardless of malignancy[3,5].

The present case was consistent with hibernoma in that, histologically, the mass had a lobular appearance and tumor cells were round to polygonal in shape with abundant, eosinophilic, foamy to multivacuolated cytoplasm containing lipid droplets and centrally located nuclei[3,5]. Ultrastructurally abundant, round to oval mitochondria with transverse closely-packed christae, intramatrical dense bodies and various numbers of non-coalescing vacuoles in cytoplasm[4–7] and immunohistochemically positive staining for UCP-1[8-11] further supported the diagnosis of hibernoma.

In addition, vascular invasion in the tumor capsule, cellular atypia, frequent mitoses, necrotic foci and hemorrhage were observed. Local invasion of surrounding tissues or metastases were not observed. Invasive growth, metastases, cellular pleomorphism and increased mitotic activity are all histological characteristics of malignant hibernoma[12]. Previously reported malignant hibernomas in rats were diagnosed as "malignant" based on metastasis to the lung or evident local tissue invasion[3,4]. Although metastases or local invasion of surrounding tissue were not observed, the present case was considered to be a malignant hibernoma.

UCP-1, one of the mammalian thermogenic mitochondrial proteins, is specifically expressed in brown adipocytes[8–11]. In the present case, the neoplastic brown adipocytes were positive for UCP-1 regardless of cellular atypism, suggesting that immunohistochemical evaluation for UCP-1 can be an effective diagnostic tool for hibernoma even for malignant cases.

This was a rare case of malignant hibernoma occurring spontaneously in a Sprague Dawly rat.

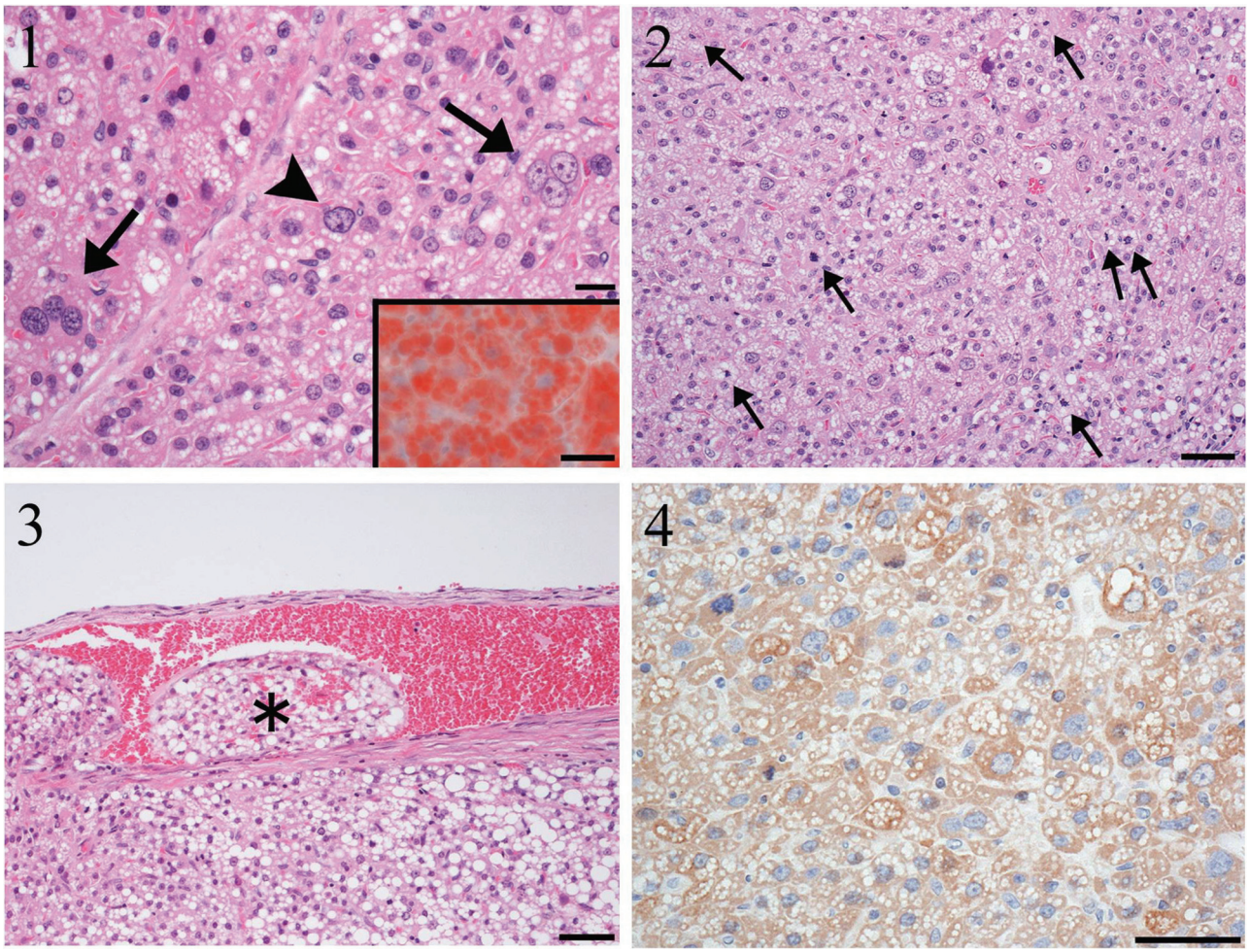

Fig. 1. Tumor cells with cellular atypia. Aggregation of tumor cells with anisocytosis, irregular shape, higher nuclear-cytoplasmic ratio, anisokaryosis, karyomegaly (arrowhead) and multinucleation (arrows). H&E. Inset: Vacuoles in the cytoplasm are positive for oil red O. Oil red O stain. Bar=20 μm.

Fig. 2. The area composed of atypical tumor cells with frequent mitoses (arrows). H&E. Bar=50 μm.

Fig. 3. Vascular invasion in the tumor capsule. Intra-capsular slit lined by endothelial-like cells filled with erythrocytes contains a small cluster of tumor cells (*). H&E. Bar=50 μm.

Fig. 4. Immunohistochemical staining for UCP-1. Tumor cells with atypia are also positive for UCP-1 in the area where neoplastic cells show frequent mitoses and pleomorphism. Bar=50 μm.

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