Case Report

Spontaneously Occurring Subcutaneous Tissue Sarcoma in a Sprague-Dawley Rat

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Abstract: A large, well-demarcated solitary mass appeared in the chest of an intact female Sprague-Dawley rat. The tumor cells were generally small and round, with scant eosinophilic cytoplasm, and arranged in an alveolar pattern. In some areas, the tumor cells contained fine vacuoles, which were confirmed to be lipid droplets. Immunohistochemistry analysis revealed that most of the tumor cells were strongly positive for vimentin and weakly positive for S100 protein, neuron-specific enolase, and CD34. The cells were negative for desmin, myogenin, α -smooth muscle actin, keratin, CD68, and von Willebrand factor. Electron microscopy revealed that there were no cellular organelles showing specific differentiation, other than mitochondria and rough endoplasmic reticulum. The tumor had no characteristic features for determining the cell of origin and was diagnosed as an undifferentiated mesenchymal tumor and classified as a sarcoma, NOS (not otherwise specified). (*J Toxicol Pathol* 2007; **20**: 161–166)

Key words: sarcoma, NOS, undifferentiated mesenchymal tumor, rat

Histopathological diagnosis of soft tissue tumors is difficult, because the appearance of different tumors may be similar. For example, fasciculated spindle cells are observed in schwannoma, fibrosarcoma, leiomyosarcoma, rhabdomyosarcoma (spindle cell type), and synovial sarcoma, whereas round cells are common in rhabdomyosarcoma (alveolar and embryonal type), liposarcoma, chondrosarcoma, osteosarcoma, and other tumors¹. Recent advances in the science of immunohistochemistry, including an increasing number of antibodies and the refinement of techniques, have allowed it to become a powerful diagnostic tool. However, tumors lacking any specific differentiation are still unavoidably diagnosed as sarcoma, NOS (not otherwise specified). Such was the case of the spontaneously occurring tumor described herein, which was found in a Sprague-Dawley rat.

A large, solitary, subcutaneous mass was noted in the thoracic region of a 42-week-old female Crj:CD (SD) rat. Before necropsy, the animal had been used for breeding, without any treatment, as indicated in the institutional guidelines of Astellas Pharma Inc. for the care and use of laboratory animals. The mass was approximately 10×7×5 cm in size, covered with connective tissue, and well

Fig. 1. Macroscopic appearance of the mass. Solitary tumor formed in the subcutaneous thoracic region (arrow), approximately 10×7×5 cm, covered by connective tissue and easily detached from the skin and surrounding tissue.

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Fig. 2. H&E staining of the mass. A) Alveolar arrangement of the tumor cells. B) Higher magnification of A: tumor cells are small and round with little eosinophilic cytoplasm. C) Highly cellular area. D) Higher magnification of C: the tumor cells contain fine vacuoles (scale bar = 100 μ m).

demarcated from the skin, skeletal muscle, and surrounding tissue (Fig. 1). When the mass was exposed, gelatinous dark red, white and brownish yellow lobes were observed.

The mass as well as other major organs and tissues were fixed in phosphate-buffered 10% formalin, and then embedded in paraffin. A portion of the formalin-fixed mass was also submerged overnight in 10% sucrose solution, and then embedded in O.C.T. compound (Sakura Finetechnical Co. Ltd., Tokyo, Japan) for frozen section. Sections were prepared from all paraffin blocks, which were then subjected to hematoxylin and eosin (H&E) staining for microscopic examination. Sections from the mass were also stained with oil red-O (OR-O, using frozen sections), alcian blue (AB), and Watanabe's silver impregnation. Immunohistochemical examination using the Envision method was conducted with the antibodies described in Table 1 [Envision method: staining performed with an Envision kit (Dako Cytomation, California, U.S.A.); detection with Envision+ (Dako Cytomation, California, U.S.A.), a biotin-free detection system that consists of a secondary antibody covalently linked to peroxidase-coated dextrose polymers; chromogen

with 3, 5'-diaminobenzidine (Muto Pure Chemicals Co. Ltd., Tokyo, Japan)]. For electron microscopic examination, the formalin-fixed mass was immersed in a solution of phosphate-buffered 2.5% glutaraldehyde and 2% paraformaldehyde for one day, and then in 1% osmium tetroxide. The tissue samples were embedded in epoxy resin and cut using a diamond knife with a Leica Ultracut S microtome (Hitachi High-technologies Corp., Tokyo, Japan). Ultrathin sections were mounted on Cu/Rh grids, stained with uranyl acetate and lead citrate, and observed under a transmission electron microscope (H-600, Hitachi High-technologies Corp., Tokyo, Japan).

Histopathologically, the tumor cells were generally small and round with scant eosinophilic cytoplasm, and arranged in an alveolar pattern (Fig. 2A-B). There were highly cellular areas consisting of tumor cells with fine vacuoles in their cytoplasm (Fig. 2C-D). OR-O staining confirmed that the vacuoles were lipid droplets (Fig. 3A). The mass contained abundant blood vessels, extensive areas of necrosis, and myxoid interstitium (Fig. 3B). Each tumor cell was surrounded by fine argentophilic fibers (Fig. 3C).

Table 1. Immunohistochemistry Antibodies

Specificity	Antibody	Manufacturer	Clone	Antigen source	Monoclonal/ Polyclonal
Mesenchymal cell	Vimentin	Nichirei Corp., Tokyo, Japan	V9	Human vimentin	Mono
Muscle cell	Desmin	MEDAK, Munich, Germany	MDE II	Human leiomyoma cells	Mono
Differentiating striated muscle cell	Myogenin	Abcam, Cambridge, U.K.	F5D	Rat recombinant myogenin amino acids 30-224	Mono
Smooth muscle cell	α -Smooth Muscle Actin	Dako A/S, Glostrup, Denmark	1A4	Synthetic α smooth muscle actin	Mono
Glial cell, melanocyte, lipocyte, striated myocyte, fibroblast	S100 protein	Dako A/S, Glostrup, Denmark	Code No. Z0311	Bovine brain S100	Poly
Neuron, nerve fiber, endocrine cell	Neuron Specific Enolase	Dako, CA, U.S.A.	Code No. L1830	Bovine brain enolase of $\gamma\gamma$ homodimer	Poly
Epithelial cell	Keratin	Dako, CA, U.S.A.	Code No. Z0622	Bovine muzzle epidermal keratin subunits of 8, 14 and 18	Poly
Macrophage (infiltrating or tissue-specific)	CD68	Chemicon International, California, U.S.A.	ED1	Rat spleen cells	Mono
Pericyte, vascular endothelial cell, hematopoietic progenitor cell	CD34	Santa Cruz Biotechnology Inc., California, U.S.A.	Code No. sc-9095	Human CD34 amino acids 151-290	Poly
Vascular endothelial cell	von Willebrand Factor	Dako A/S, Glostrup, Denmark	A0082	Human plasma	Poly

Table 2. Immunohistochemistry Summary

Antibody	Mass		Normal tissue or positive control
	Tumor cells	Blood vessels and others	
Vimentin	++	++ (vascular endothelial cell)	
Desmin	–	++ (vascular smooth muscle)	++ (vascular smooth muscle and skeletal muscle)
Myogenin	–	–	++ (positive control: skeletal muscle of rat day 18 embryo)
α -Smooth Muscle Actin	–	++ (vascular endothelial cell)	++ (vascular smooth muscle and endothelial cell)
S100 protein	+	–	+ (lipocyte)
Neuron Specific Enolase	+	–	++ (normal nerve bundle)
Keratin	–	–	++ (positive control: cornified epithelium of the normal forestomach)
CD68	–	+	
CD34	+	+	
von Willebrand Factor	–	+	

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

Mitotic figures were also abundant. Based on the histopathological appearance, the possibilities of rhabdomyosarcoma (round tumor cells with eosinophilic cytoplasm in an alveolar pattern), adipocytic tumor (lipid droplets containing tumor cells), and vascular tumor (abundant blood vessels) were presented. Therefore

immunohistochemistry and electron microscopy were conducted for diagnosis.

Immunohistochemical analysis was performed. First, anti-vimentin and keratin staining were utilized to determine whether the tumor origin was mesenchymal or epithelial. Most of the tumor cells were strongly positive for vimentin

Fig. 3. Specific staining of the mass. A) OR-O staining of the tumor cells with fine vacuoles. B) AB staining of the myxoid interstitium. C) Watanabe silver staining of the typical alveolar pattern (scale bar = 100 μ m).

(Fig. 4A) and negative for keratin; therefore, the tumor was confirmed to be mesenchymal in nature. Second, to examine muscular differentiation, anti-desmin, myogenin, α -smooth muscle actin staining were performed. The result was negative for all of these examination; therefore, the possibility of skeletal or smooth muscle tumors was eliminated. Furthermore, anti-von Willebrand factor and

Fig. 4. Immunohistochemistry of the mass: A) vimentin, B) CD68, and C) S100 protein staining (scale bar = 100 μ m). Tumor cells are positive for vimentin and S100 protein, while only scattered macrophages are positive for CD68.

CD68 staining were performed to investigate vascular and histiocytic orientation, respectively. Negative results (Fig. 4B) excluded both possibilities. Most of the tumor cells were weakly positive for S100 protein (Fig. 4C), neuron-specific enolase and CD34; however, the significance of these results was unclear. The results are summarized in

Fig. 5. Electron microscopy of the mass: A) toluidine blue staining (scale bar = 100 μ m), B) higher magnification of A ($\times 4,000$), C) & D) lipid droplets and distended endoplasmic reticulum ($\times 3,500$ and $\times 10,000$, respectively).

Table 2.

An electron microscopic study was performed to examine the cytoplasmic components and the presence of lipoblasts. The tumor cells contained a few organelles such as mitochondria and rough endoplasmic reticulum (Fig. 5A-B), which were thought to correspond to the eosinophilic cytoplasm, and had a high nucleus/cytoplasm ratio. There were no myofibrils, secretory granules, or basement membrane in any tumor cells, which would have allowed specific differentiation. A small number of tumor cells

contained a few lipid droplets (Fig. 5C-D); however, there were no cells filled with lipid droplets, which could be considered to be lipoblasts. No ultrastructural finding showed any diagnostic basis of the tumor differentiation.

In the present case, a large subcutaneous solitary mass was observed spontaneously in a female SD rat. It was a mesenchymal, not an epithelial tumor, on the basis of positive vimentin and negative keratin immunohistochemistry. The histopathological finding that most tumor cells were round with eosinophilic cytoplasm

Table 3. Nomenclature of Rat Soft Tissue and Musculoskeletal Tumors

Cell origin	Tumor Classification [Type]	
	Benign	Malignant
Blood vessel	Hemangioma [capillary; cavernous]	Hemangiosarcoma
Pericyte of blood vessel	Hemangiopericytoma, benign	Hemangiopericytoma, malignant
Lymph vessel	Lymphangioma	Lymphangiosarcoma
White adipocyte	Lipoma [angiolipomatous; myxolipomatous]	Liposarcoma [pleomorphic]
Brown adipocyte	Hibernoma, benign	Hibernoma, malignant
Fibroblast	Fibroma [durum; fibromatosis type; molle; myxomatous]	Fibrosarcoma [myxomatous]
Histiocyte	Fibrous histiocytoma, benign	Fibrous histiocytoma, malignant [fibrous type; myxoid; pleomorphic]
Smooth muscle cell	Leiomyoma	Leiomyosarcoma
Skeletal muscle cell		Rhabdomyosarcoma
Schwann cell	Schwannoma, benign	Schwannoma, malignant
Granular cell	Granular cell tumor, benign	
Neuroendocrine cell	Neuroendocrine cell tumor, benign	Neuroendocrine cell tumor, malignant
Stem cell	Teratoma, benign	Teratoma, malignant
Undifferentiated mesenchymal cell		Sarcoma, NOS
Undifferentiated mesenchymal cell and others		Sarcoma, mixed
Epidermal cell		Carcinoma, NOS
Unclassifiable	Unclassifiable tumor, benign	Unclassifiable tumor, malignant

arranged in an alveolar pattern indicated rhabdomyosarcoma; however, the results that tumor cells were negative for desmin and myogenin immunohistochemistry and contained no myofibrils in the electron microscopy excluded this possibility. Fine lipid droplets in the tumor cells suggested an adipocytic tumor. However, this possibility was also denied because no lipoblasts were observed under electron microscopy. Similarly, vascular tumor (indicated by abundant blood vessels) and histiocytic tumor were excluded because of von Willebrand factor and CD68 negative immunohistochemistry results. No cytoplasmic components indicating the specification were seen under electron microscopy, and the significance of the result that most of the tumor cells were weakly positive for S100 protein, neuron-specific enolase and CD34 in the immunohistochemistry was unclear. To sum up, there were no evidence to show any specific differentiation in all the examinations, and the mass was thought to be an undifferentiated mesenchymal tumor. Classifications of soft tissue tumors have been published previously^{2,3}. According to definitions issued by the international harmonization of rat nomenclature, shown in Table 3 (2000, Department of Information Technology / Pathology of the Fraunhofer

Institute of Toxicology and Aerosol Research, Hannover, Germany <http://www.toxpath.org/nomen/index.htm>), this undifferentiated mesenchymal tumor was classified as a NOS sarcoma.

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