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Case Report

Spontaneous hemangiosarcoma in the spleen and liver of a young rat

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Abstract: Spontaneous hemangiosarcoma in young rats is rare. In this report, we describe a case of a spontaneous hemangiosarcoma in the spleen and liver of young rats. At necropsy, multiple pale red masses were observed in the spleen. Histopathologically, solid growth and haphazardly arranged neoplastic cells were observed, although no characteristic growth pattern was observed. In contrast, irregularly sized small slit-shaped spaces containing erythrocytes were found among the neoplastic cells. Reticular fibers incompletely surrounding the neoplastic cells were observed by silver staining. Immunohistochemistry revealed that the neoplastic cells were positive for vWF and CD34. Electron microscopic examination revealed that the neoplastic cells had erythrocytes in the lumen and Weibel-Palade bodies in the cytoplasm and were arranged along a discontinuous basal lamina. These features indicate that the tumor originated from vascular endothelial cells. Based on these results, the tumor was diagnosed as a hemangiosarcoma in the spleen and liver. (DOI: 10.1293/tox.2021-0042; J Toxicol Pathol 2022; 35: 89–93)

Key words: hemangiosarcoma, rat, spleen, liver, young, tumor

Hemangiosarcoma is commonly found in rodents of laboratory animals[1] and occurs more frequently than hemangiomas and in mice than in rats. The liver and spleen have been reported as the most common primary sites by a number of surveys analyzing 2-year bioassay data for control animals[2–4]. The incidence of spontaneous hemangiosarcoma in Sprague-Dawley (SD) rats is 0% (female) and 0.36% (male) in the spleen and 0% (male) and 0.09% (female) in the liver[5]. In addition, it has been reported that spontaneous hemangiosarcoma is commonly found in rats aged 91 weeks or older[6]. Only a few cases have been reported in young rats[7–9]. In this report, we elucidate the occurrence of spontaneous hemangiosarcoma in the spleen and liver of young rats and describe the histological, immunohistological, and ultrastructural features of this tumor.

Animal experiments were approved by the Committee on Animal Experiments of Otsuka Pharmaceutical Factory, Inc. Three-week-old male Crl:CD (SD) rats (Charles River Laboratories Japan, Inc., Kanagawa, Japan) were purchased for technique acquisition training. The animals were housed in groups of three under a controlled environment: 20–26°C temperature range, 40–70% humidity range with 13–16 air changes/h, and a photoperiod of 12 h nominal. At the age of 7 weeks, the animals showed a normal appearance, were euthanized by exsanguination under anesthesia, and were subjected to necropsy. At necropsy, multiple pale red masses measuring 0.3–1 cm were found on the spleen (Fig. 1). The masses were soft, the surface was smooth and shiny, the cut surface was pale red and solid, and each mass showed a distinct margin. No gross abnormalities were observed in any other organs. Blood tests were not performed.

The spleen, liver, and other organs were fixed in 10% volume by volume (v/v) neutral buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin. Additionally, sections from the spleen were stained using Watanabe's silver stain for the reticulum. Immunostaining with vimentin, von Willebrand factor (vWF), CD34, proliferating cell nuclear antigen (PCNA), cytokeratin wide spectrum (CK), podoplanin, CD79a, CD3, and Iba1 was also performed (Table 1). For electron microscopy, samples were picked from the formalin-immersed spleen tissue and then fixed in phosphate buffered 1% osmium tetroxide, embedded in epoxy resin, and ultra-sectioned. The sections were double-stained with uranyl acetate-lead citrate. They were then examined using a transmission electron microscope (H-7800TEM, Hitachi High-Technologies Co, Ltd, Tokyo, Japan).

Most of the normal tissue in the spleen was replaced by multicentric neoplastic tissue (Fig. 2A). The tumor comprised neoplastic cells that proliferated solidly and grew nodularly (Fig. 2B) while compressing the surrounding tissue. No collagenous fibers were observed between the neoplastic and normal tissues, and the margins were unclear. Neoplastic cells had poorly basophilic cytoplasm and were

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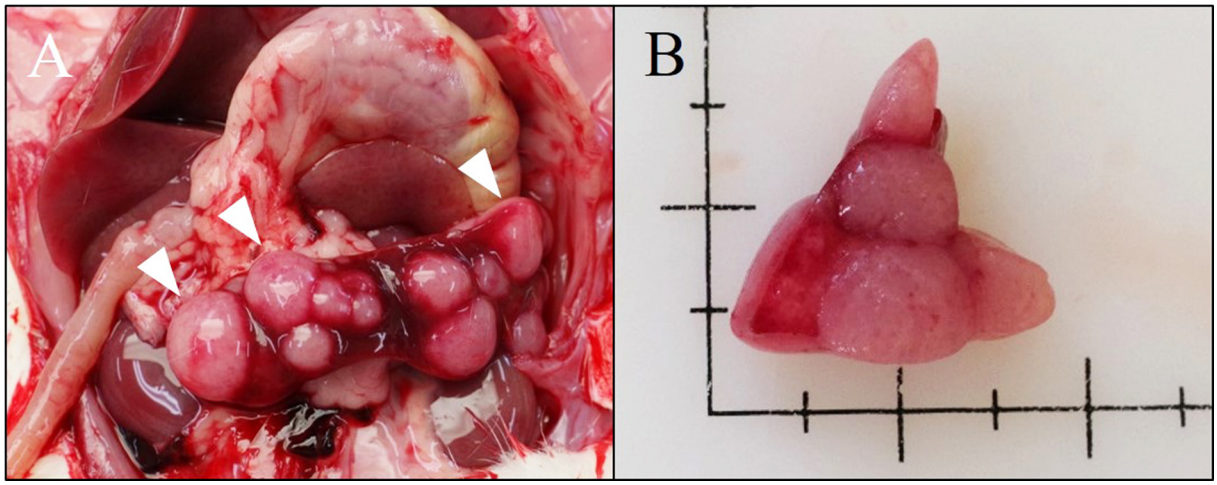

Fig. 1. Macroscopic findings. A, B. Multiple pale red masses measuring 0.3–1 cm (arrowheads) in the spleen.

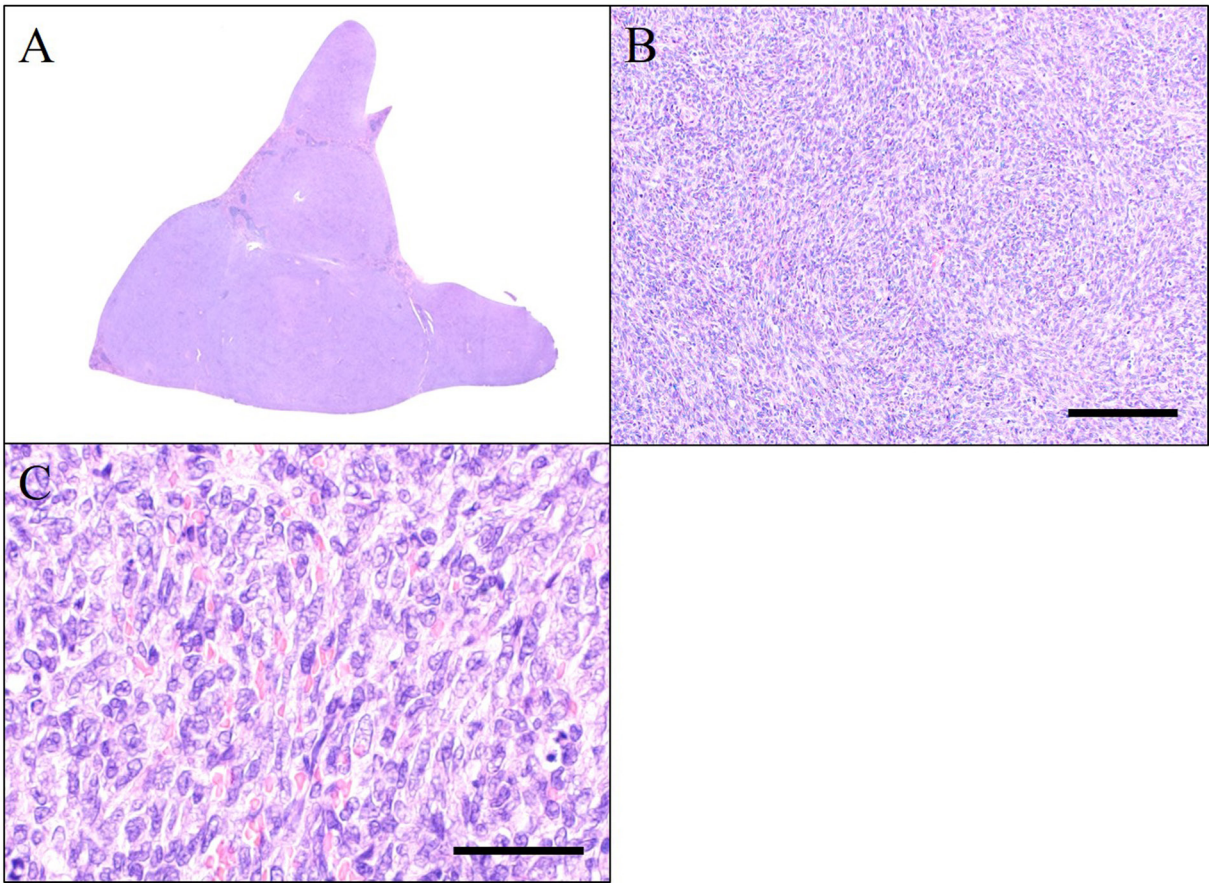

Fig. 2. Microscopic findings (hematoxylin and eosin). A. Most of the normal tissue of the spleen is replaced by multicentric neoplastic tissue. B. The tumor comprises neoplastic cells that proliferated solidly and grew nodularly. Bar=200 μm. C. Neoplastic cells with a poorly basophilic cytoplasm and spindle-shaped to irregularly rounded nuclei with scattered chromatin and occasional nucleoli. In addition, irregularly sized slit-shaped small cavities are observed among the neoplastic cells, and some of them contain erythrocytes. Bar=50 μm.

spindle-shaped, with irregularly rounded nuclei containing scattered chromatin and occasional nucleoli (Fig. 2C). Mitotic figures were occasionally observed. Solid growth and haphazardly arranged neoplastic cells were observed in the neoplastic tissue, although no characteristic growth pattern was observed. Irregularly sized slit-shaped small spaces

Table 1. Immunohistochemical Reactivity of Antigens in the Splenic Tumor

Primary antibody	Reactivity	Dilution	Host	Manufacturer, clone (product code)	Antigen retrieval
Vimentin	+	1:100	Mouse	Dako, V9	Citrate buffer, pH 6.0, 121°C 10 min
vWF	+	1:2,000	Rabbit	Abcam, ab6994	Proteinase-K, 10 min
CD34	+	1:200	Rabbit	Boster Biological Technology, PA1334	Citrate buffer, pH 6.0, 121°C 20 min
PCNA	+	1:6,000	Rabbit	Abcam, ab18197	Citrate buffer, pH 6.0, 121°C 10 min
CK	−	1:500	Rabbit	Dako, Z0622	Proteinase-K, 10 min
Podoplanin	−	1:400	Mouse	AngioBio, 11-035	Citrate buffer, pH 6.0, 121°C 10 min
CD79a	−	1:250	Mouse	Abcam, HM57	
CD3	−	1:50	Mouse	Dako, F7.2.38	Tris/EDTA buffer, pH 9.0, 121°C 20 min
Iba1	−	1:5,000	Rabbit	Wako Chemicals, 019-19741	Citrate buffer, pH 6.0, 121°C 10 min

Grade of findings; −, negative; +, positive.

were found among the neoplastic cells, and some of them contained erythrocytes (Fig. 2C). Infiltration of neoplastic cells into the splenic pulp vein was also observed at the margin of the neoplastic tissue. In addition, in the stroma, macrophage-like cells with irregular nuclei, such as cuts and beans, and lymphocyte-like cells with relatively small and round nuclei and rich chromatin were observed. Histopathological examination of all the organs revealed that cells similar to neoplastic cells observed in the spleen were found to grow like small foci in the sinusoids of the liver and were accompanied by the formation of slit-shaped small spaces containing erythrocytes.

In Watanabe's silver stain to study the reticulum, an image was observed in which reticular fibers incompletely surrounded the neoplastic cells inside the neoplastic tissue (Fig. 3A). Table 1 shows the results of immunohistochemical staining. The neoplastic cells were positive for vimentin, vWF (Fig. 3B and 3C), and CD34, a marker of vascular endothelial cells. However, they were negative for CK, podoplanin (Fig. 3D), CD79a, CD3, and Iba1. Macrophage-like cells in the stroma were positive for Iba1, and lymphocyte-like cells, which were considered macrophages and lymphocytes that infiltrated into the neoplastic tissue, were positive for CD3. The majority of neoplastic cells were positive for PCNA. Neoplastic cells observed in the liver were positive for vWF and negative for podoplanin, similar to the neoplastic cells in the spleen. Examination of transmission electron microscopy of the splenic tumor revealed that the neoplastic cells had erythrocytes in the lumen and Weibel-Palade (WP) bodies, which are special granules of vascular endothelial cells and characterized by a single membrane and dense interior with rod-shaped profiles, in the neoplastic cytoplasm and were arranged along a discontinuous basal lamina (Fig. 4).

Grossly, hemangiosarcoma is recognized as a nodular lesion[5]; and the color is red, reddish brown, or black and may be multicentric[10]. Histopathologically, atypical endothelial cells form vascular channels and solid cellular masses supported by variably developed fibrovascular stroma. Neoplastic cells are commonly spindle-shaped, but can also be round, polygonal, or extremely irregular in form, with single or multiple irregularly shaped and sized nuclei that are often large and lobulated with large masses of chromatin[1]. Hemangiosarcoma is positive for vimentin, vWF, and CD34 specific to endothelial cells and negative for podoplanin specific to lymphatic endothelium[8, 11]. Neoplastic cells are surrounded by silver fibers, and the diagnosis is based on the presence of WP bodies on electron microscopy[5].

In this case, no clear luminal structure containing erythrocytes, which was histopathologically found in hemangiosarcoma, was observed. Undifferentiated sarcoma-like morphology with poor histological features in most areas of the tumor tissue, with small spaces containing erythrocytes, were observed between the neoplastic cells, which was thought to indicate that the neoplastic cells formed a vascular-like structure. Therefore, a routine histopathological diagnosis was considered difficult. Since the neoplastic cells were positive for vimentin, vWF, and CD34 and negative for podoplanin, they were considered to be tumors originating from the vascular endothelium. In addition, they were recognized as findings reflecting the origin of neoplastic cells: cytoplasmic WP bodies, which are morphological images specific to the vascular endothelium, multiple neoplastic cells surrounded by reticular fibers that imitate the vascular structure, and neoplastic cells arranged along a discontinuous basal lamina with erythrocytes in the lumen. Neoplastic cells are considered to be lesions in the category of malignant tumors because they exhibit cellular atypia and high proliferative activity, infiltrate surrounding tissues and blood vessels, and can cause distant metastasis to the liver.

The fact that it was recognized as a pale red mass rather than a red mass characteristic of macroscopic hemangiosarcoma was considered to be correlated with the low degree of histopathological differentiation. Regarding the pathogenesis, the lesion was found as a multiple mass in the spleen, and histopathological examination of the other organs revealed only a small growth foci in the liver; thus, the spleen was considered to be the primary lesion. Infiltration of the neoplastic cells into the vein of the splenic pulp was observed, indicating that multicentric lesions were formed in the spleen via intravascular metastasis from the primary lesion and distant metastasis to the liver.

There have been two reports of hemangiosarcoma in the spleen and/or liver of young rats. In one report, splenic

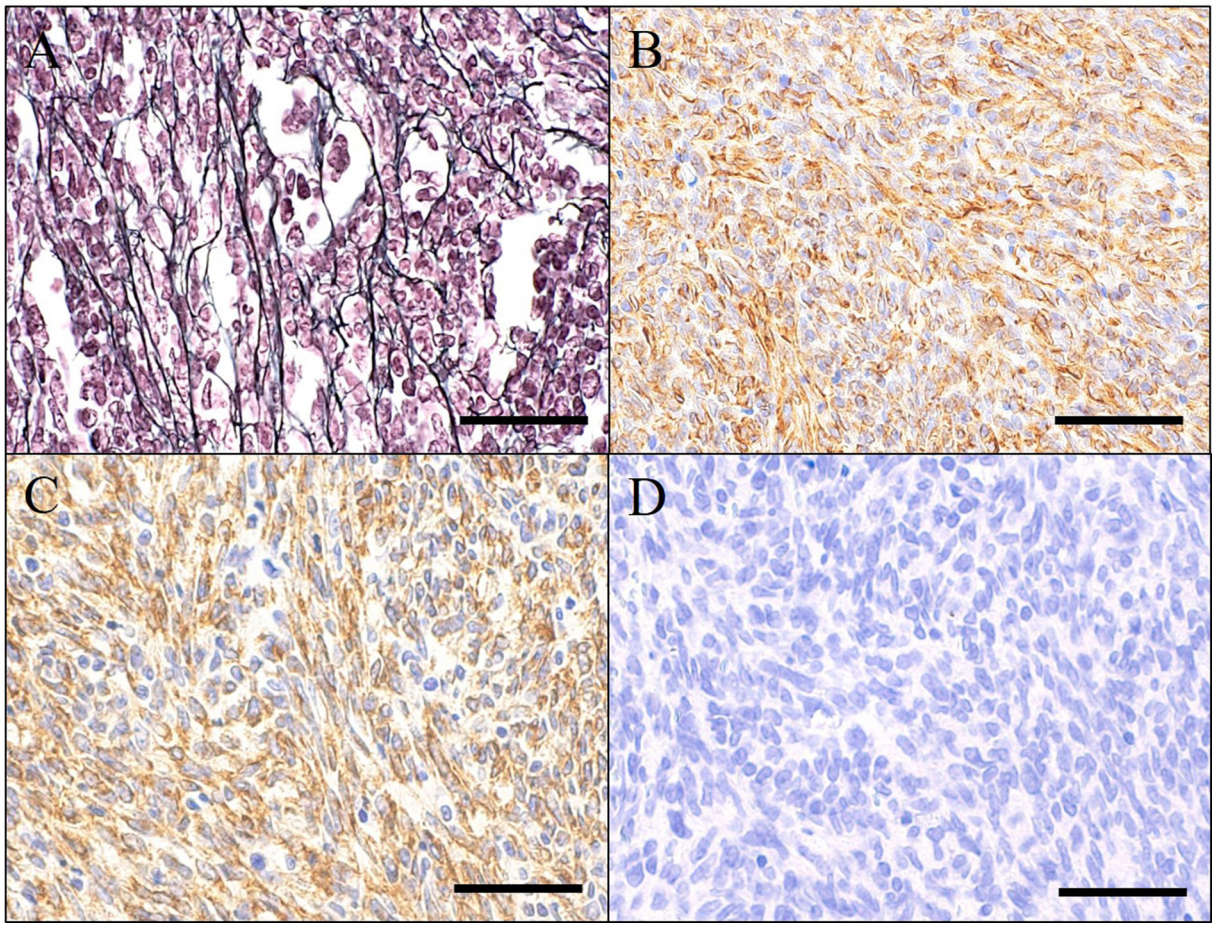

Fig. 3. Microscopic findings (immunohistochemistry and special staining). A. In the Watanabe's silver stain for the reticulum, an image is observed in which argentophil fibers incompletely surround the neoplastic cells inside the neoplastic tissue. Bar=40 µm. B. The neoplastic cells are positive for vimentin. Bar=50 µm. C. The neoplastic cells are positive for von Willebrand factor. Bar=50 µm. D. The neoplastic cells are negative for podoplanin. Bar=50 µm.

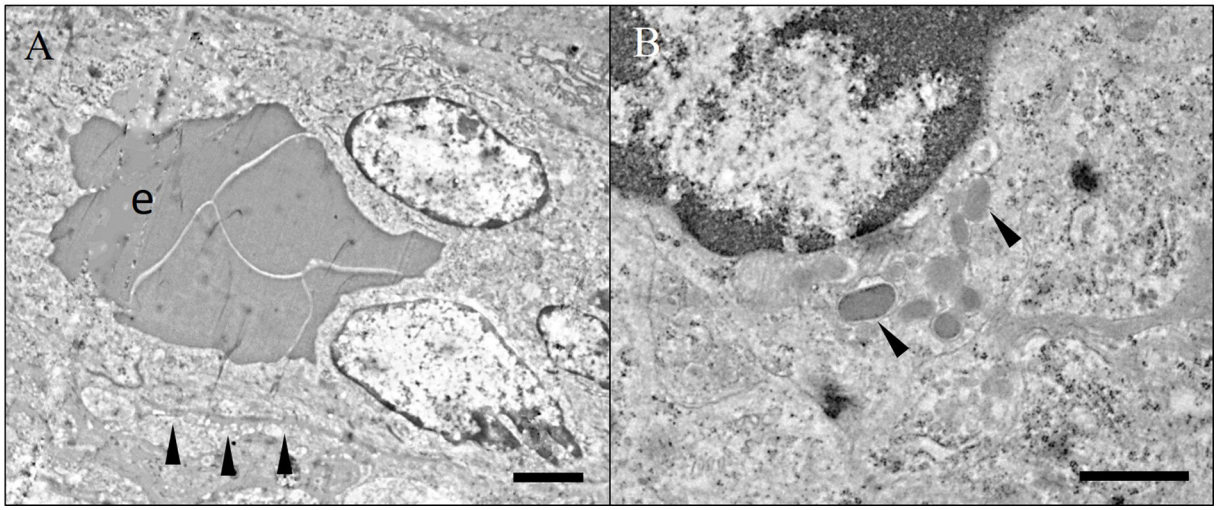

Fig. 4. Ultrastructual findings (transmission electron microscopy). A. Neoplastic cells with erythrocytes in the lumen, arranged along a discontinuous basal lamina (arrowheads). e, erythrocytes. Bar=2 µm. B. Neoplastic cells with Weibel-Palade bodies (arrowheads), characterized by a single membrane and dense interior with rod-shaped profiles, in the neoplastic cytoplasm. Bar=1 µm.

hemangiosarcoma was observed in a 9-week-old SD rat, comprising a dense sheet of pleomorphic cells frequently observed in undifferentiated tumors and intravascular metastasis to the liver[8]. In another report, similar histological features were observed in metastasized hepatic hemangiosarcoma of a 7-week-old SD rat[9]. The morphological features of these case reports, including this case, reflect the unique characteristics of hemangiosarcoma in young SD rats: extremely undifferentiated histological images without formation of clear vascular structures. In other cases of a young Wister rat[7] and older SD rats[6], comparatively mature blood vessels were observed, which supports this hypothesis.

In this study, the tumor was considered to be a hemangiosarcoma due to its histological, immunohistological, and ultrastructural features. Further investigations are required to clarify the characteristics of hemangiosarcoma in young rats.

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

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