

Case Report

Metastatic hemangiosarcoma of the liver in a young rat

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Abstract: Spontaneous hemangiosarcoma is generally uncommon in rats. Furthermore, there are only a few case reports in young rats. This report describes a spontaneous hemangiosarcoma in a young 7-week-old rat. At necropsy, no remarkable changes were noted in any organs including the liver. Histopathologically, multifocal small tumors were located mainly in the perilobular region of the liver. The tumors comprised polygonal to short spindle-shaped cells that showed cellular atypia and local infiltration. In the foci, there were blood-filled spaces lined by spindle-shaped cells. Reticular fibers, which were loosely connected together and formed an irregular network within the foci, were noticed with Azan and silver stain. Immunohistochemistry revealed that tumor cells expressed vascular endothelial cell markers: von willebrand factor and CD34, respectively. These features indicate that the tumor originated from vascular endothelial cells. Although the primary lesion was uncertain, the foci were all small and multicentrically located mainly in the perilobular region, indicating that the liver lesion likely formed by hematogenic metastasis. Taken together, we diagnosed this case as a metastatic hemangiosarcoma. (DOI: 10.1293/tox.2016-0040; J Toxicol Pathol 2017; 30: 75–78)

Key words: rat, tumor, spontaneous, hemangiosarcoma, metastatic, liver

Hemangiosarcoma is defined as a malignant tumor arising from endothelial cells of blood vessels¹. In rodents, a hemangiosarcoma can be experimentally induced by using many substances: vinyl chloride², PPAR agonists³, butoxyethanol^{4, 5}, and 1,3 butadiene⁶. Spontaneous hemangiosarcoma is a rare tumor, and its incidence in Sprague-Dawley rats is reported to range from 0.3% (female) to 0.4% (male)⁷. There are only a few case reports^{8, 9} on young rats. Although the spleen is reported to be the most frequent site of the primary lesion in rats⁷, the tongue⁸, pancreas¹⁰, lung¹¹, and mesenteric lymph node¹² have also been reported as sites of primary lesions. Metastasis is often present¹, and cases of distant metastasis have also been reported^{8, 9}. In this paper, we report a spontaneous case of metastatic hemangiosarcoma in a young rat and describe histopathological and immunohistochemical features of the tumor.

This animal experiment was reviewed and approved by the Institutional Animal Care and Ethics Committee of Otsuka Pharmaceutical Factory. The experimental protocol was conducted according to the relevant guidelines. The animal, a 3-week-old male Slc:SD rat (Sprague-Dawley derived) was purchased from Japan SLC Inc. (Shizuoka, Japan)

for a food control group in a pharmacological study. This animal was housed in a wire mesh cage (5 rats per cage) in a room maintained under the following conditions: $23 \pm 3^\circ\text{C}$, $55 \pm 15\%$ humidity, and a 12-h light-dark cycle. At the age of 7 weeks, the animal showed a normal appearance, was anesthetized with isoflurane, and was euthanized by exsanguination. At necropsy, no remarkable changes were noticed in the liver and other organs. Furthermore, serum AST and ALT levels were within the normal range. For microscopic examination, a liver sample was collected from the left lateral lobe and fixed in phosphate buffered 10% formalin. The sample was then embedded in paraffin, sectioned at $4 \mu\text{m}$, and stained with hematoxylin and eosin (H&E). In addition, Azan stain and Watanabe's silver stain were applied for confirmation of reticular fibers. Immunohistochemical staining was conducted with antibodies for vimentin, proliferating cell nuclear antigen (PCNA), von willebrand factor (vWF), CD34, and podoplanin (Table 1). All sections were incubated at 4°C overnight and then incubated with an EnVision system (EnVisionTM + System-HRP labelled polymer, Dako, Tokyo, Japan) for 30 minutes at room temperature. Sections were visualized with diaminobenzidine and counterstained with hematoxylin.

Histopathologically, multifocal tumors were seen in the liver parenchyma as basophilic small foci located mainly in the perilobular region (Fig. 1a). Tumor cells formed small nests and sheet patterns in the sinusoid with no encapsulation, which compressed the surrounding hepatocytes, and hepatocellular necrosis was seen sparsely. Tumor cells were polygonal to short spindle-shaped cells, showed a clear cell borders and high N/C ratios, and contained irregularly

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Table 1. Immunohistochemical Reactivity of Antigens in the Tumor

Primary antibody	Reactivity	Dilution	Manufacturer, clone (product code)	City, state, country	Antigen retrieval
Vimentin	++	Prediluted	Dako, V9	Glostrup, Denmark	Tris/EDTA buffer, pH 9.0, 121°C 15 min
PCNA	++	1:400	Dako, PC10		
Von Willebrand Factor	++	1:600	Abcam, ab6994	Cambridge, UK	Proteinase-K, 10 min
CD34	++	1:200	Boster Biological Technology, PA1334	Pleasanton, CA, USA	Citrate buffer, pH 6.0, 121°C 15 min
Podoplanin	-	1:400	AngioBio, 11-035	Del Mar, CA, USA	

Grade of findings; -, negative; +, positive; ++, strongly positive.

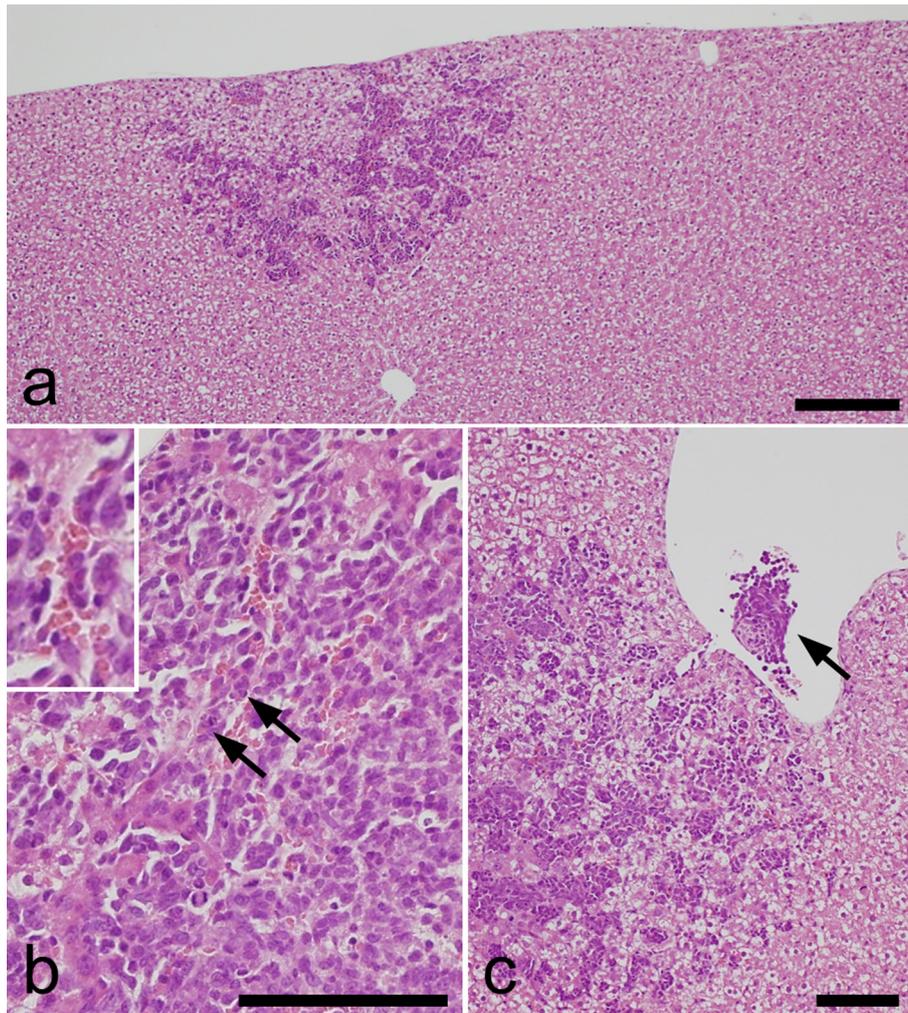


Fig. 1. (a) Tumors were seen in the liver parenchyma as basophilic small foci located mainly in the peribiliary region. Bar = 200 μ m. (b) Tumor cells showed cellular atypia and formed small nests and sheet patterns in the sinusoid with no encapsulation. Hepatocellular necrosis was seen sparsely (see the arrows). Tumor cells showed a clear cell border and formed narrow spaces containing erythrocytes (upper left inset). Bar = 100 μ m. (c) Local invasion into the adjacent blood vessel was seen (see the arrow). Bar = 100 μ m. H&E.

shaped to oval-shaped hyperchromatic nuclei. Mitotic figures were often seen. Tumor cells formed narrow spaces containing erythrocytes. Local tumor cell invasion into the adjacent blood vessels was seen (Fig. 1b, c). In the tumor cells-aggregated foci, reticular fibers, which were loosely connected together and formed an irregular network, were noticed by Azan stain and Watanabe's silver stain

(Fig. 2a, b). Immunohistochemically, tumor cells reacted with vimentin antibody and were labelled with PCNA antibody, indicating high proliferation activity. vWF and CD34, both endothelial cell markers, were also positively identified in the tumor cells (Fig. 2c-e), whereas podoplanin, a lymphatic endothelial cell marker, was negative. These results suggest that the tumor cells originated from vascular

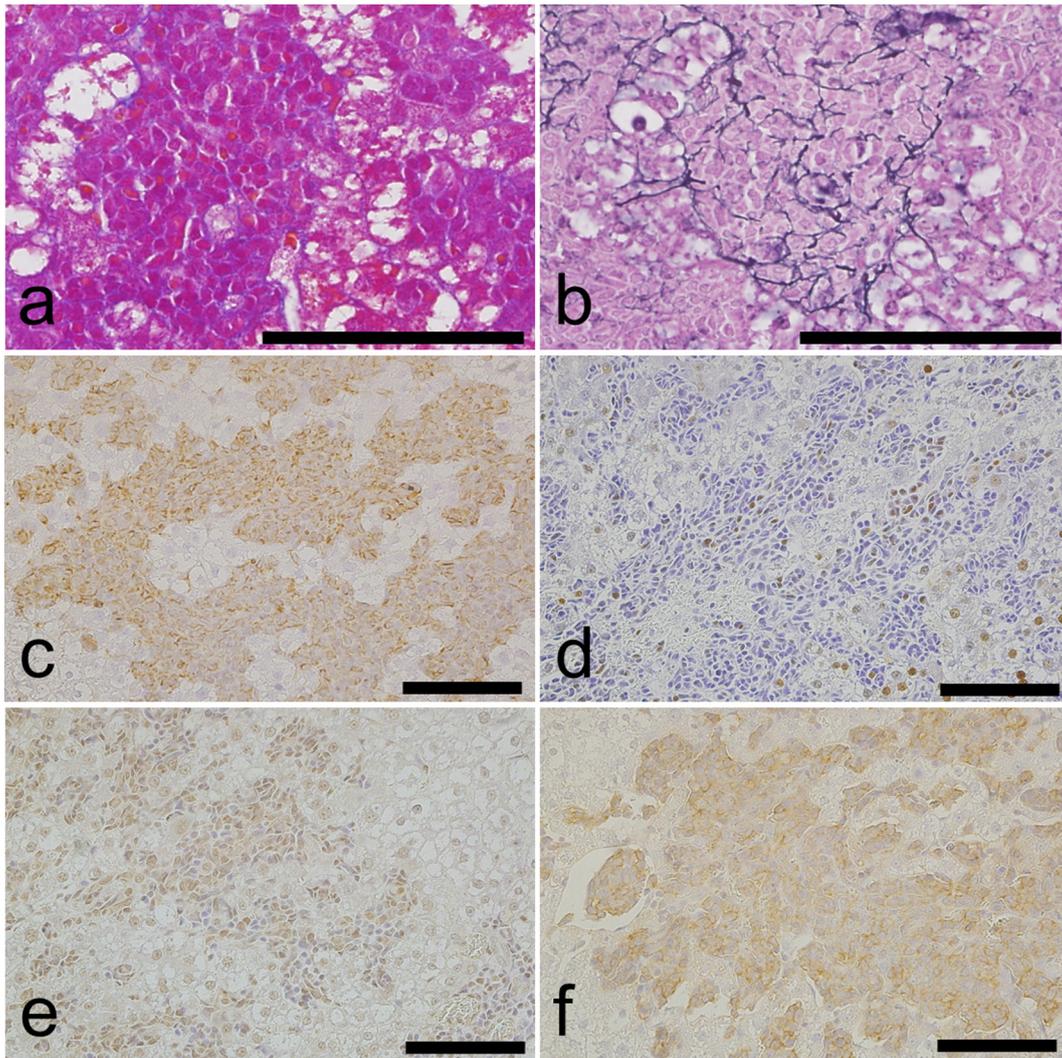


Fig. 2. (a) Azan stain. (b) Watanabe's silver stain. Reticular fibers were noticed with Azan stain (blue) and Watanabe's silver stain (black); they were loosely connected together and formed an irregular network. (c–f) Immunohistochemical reactivity of tumor cells. Tumor cells reacted with (c) vimentin, (d) PCNA, (e) vWF, and (f) CD34. Bar = 100 μ m.

endothelial cells. In this case, tumor cells showed cellular atypia, high proliferation activity, and local invasion. These characteristics are evidence of the malignant potency of the tumors. Considering the origin of the tumor cells, we diagnosed this tumor as hemangiosarcoma.

Differential points of hemangiosarcoma are discussed below. Narrow spaces contained erythrocytes that were thought to be part of an incomplete blood vessel structure. Reticular fibers that formed in the tumor cells, as documented in another case report of hemangiosarcoma^{9, 10}, could have possibly been a basal membrane formed by vascular endothelia. These findings suggest that the tumor cells had the characteristics of blood endothelial cells, which are the key features of a diagnosis of hemangiosarcoma. Immunohistochemically, labelling with vWF and CD34 indicated that the tumor cells originated from vascular endothelial cells but were not differentiated from lymphatic endothelial cells. No podoplanin, which is a mucin-type transmembrane

glycoprotein expressed in the lymphatic endothelium, but not vascular endothelial cells¹³, was identified in the tumor. This result ruled out lymphangiosarcoma or a lymphatic endothelial cell origin. Another means of differentiation is through the detection of Weibel-Palade bodies, a marker for endothelial cells¹⁴, with an electron microscopic examination; however, electron microscopic examination was not performed because of the lack of an extra tissue specimen. Hemangiosarcoma originating from liver has an irregular perimeter, which may be either raised or depressed when near the surface of the liver. Histologically, tumor cells may form solid masses, and necrosis and thrombosis are also frequently present¹⁵. These features do not correspond with this case. Moreover, the foci were all small and multicentrically located mainly in the perilobular region, indicating that the liver lesion was likely formed by hematogenic metastasis. In a case report of metastatic hemangiosarcoma⁹, which developed primarily in the spleen, tumor embolism was mainly

observed in periportal regions in the liver. The above report implies the pathogenesis of the liver lesion was hematogenic metastasis. Multifocal tumors were thought to have developed by hematogenic metastasis through a portal vein or hepatic artery from the primary site. On the other hand, the primary lesion was uncertain because hemangiosarcoma can arise from any organ containing endothelial cells and histopathological examination was done only for the liver. Despite the fact that metastatic lesions might have been present in other organs, pathological conditions seemed to be mild because systemic effects were not present; the animal showed a normal appearance, normal serum AST and ALT levels, and no remarkable macroscopic changes in any organs.

In the present case, differentiation was difficult due to the observation of fewer histological characteristics in the tumor tissue on the basis of H&E staining alone. Extramedullary hematopoiesis, leukemia, and lymphoma were all suspected because of their similarities in terms of cellular morphologies and growth patterns compared with this case. An exclusive diagnosis was made by special staining and immunostainings; extramedullary hematopoiesis and leukemia were excluded based on negative peroxidase staining and negative hemoglobin and lysozyme immunostaining, and lymphoma was excluded based on negative CD3 and CD79a immunostaining. Immunostaining with markers of vascular endothelial cells is necessary for differentiation.

Although the primary lesion was uncertain in this case, the foci were all small and multicentrically located mainly in the perilobular region, indicating that the liver lesion was likely formed by hematogenic metastasis. Spontaneous hemangiosarcoma is rare in rats. A further investigation is required to clarify the characteristics of hemangiosarcoma.

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