Case Report

Neuromuscular and vascular hamartoma of the small intestine in an F344 rat

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Abstract: An intestinal mass was found in the border area of the jejunum and ileum of a 110-week-old male F344 rat. Histopathologically, the mass protruded into the lumen and was covered with intestinal epithelium, exhibiting a normal architecture. The lesion was located in the submucosa and consisted of loose connective tissue, smooth muscle, scattered ganglion cells, and blood vessels of various sizes. Although these components showed an irregular and disordered structure, no cellular atypia, increased proliferation activity, or invasive growth to adjacent tissues were detected. Immunohistochemical analyses revealed that smooth muscle, ganglion, and endothelial cells were positive for α -smooth muscle actin and vimentin, S-100, and CD34 and von Willebrand factor, respectively, indicating maturation of these cells. Thus, the mass was diagnosed as a neuromuscular and vascular hamartoma of the small intestine. To the best of our knowledge, this is the first report of this type of lesion in rodents. (DOI: 10.1293/tox.2020-0059; J Toxicol Pathol 2021; 34: 113–117)

Key words: hamartoma, small intestine, jejunum, ileum, Fischer rat

Neuromuscular and vascular hamartoma (NMVH) is an uncommon lesion of the small intestine in humans and typically presents with nonspecific clinical signs, including abdominal pain, recurrent obstruction, or occult gastrointestinal bleeding. The first case of NMVH was reported in 1982, and at least 24 cases have since been reported^{1–6}. Most cases of NMVH were found in the submucosa of the small intestine and were histopathologically characterized by irregular proliferation of smooth muscle fascicles, unmyelinated nerve fibers with ganglion cells, and vascular structures that occasionally had a thickened wall^{3,4}. In animals, only a few cases of NMVH have been reported, such as in dogs, including a case with invagination in the cecum⁷. In this report, we describe a case of NMVH of the small intestine in a male F344 rat at 110 weeks of age.

The case was found among a group of male rats (F344/DuCrj; Charles River Laboratories, Yokohama, Japan) in the low-dose dietary administration group in a 2-year carcinogenicity study. No similar lesions of the small intestine

were observed in any other rats used in this study. Thus, the current case was considered to have a spontaneous lesion that was not related to the test chemical treatment.

The study on carcinogenicity was initiated after the rats underwent one week of acclimatization. The six-week-old animals were housed in plastic cages on hardwood chip bedding in a room with a barrier system for controlled light/dark cycles (12 h), ventilation (air exchange rate: 18 times/h), temperature ($24 \pm 1^\circ\text{C}$), and relative humidity ($55 \pm 5\%$), and allowed free access to tap water and CRF-1 basal diet (Oriental Yeast, Tokyo, Japan), with or without test chemicals for 104 weeks. The experimental design was approved by the Animal Care and Utilization Committee of the National Institute of Health Sciences, Japan, and all animals were cared for in accordance with institutional guidelines. The animals showed no clinical signs throughout the experiment and was euthanized under deep anesthesia at 110 weeks of age as scheduled. At necropsy, an intestinal mass 10 mm in diameter was detected in the lumen and at the border area of the jejunum and ileum. No scar formation, adhesion, nor protrusion were observed on the serosal surface. After removal and fixation in 10% neutral-buffered formalin, the mass was routinely embedded in paraffin, and 4- μm thick sections were cut. For histological observation, hematoxylin and eosin, and toluidine blue stainings were performed. Immunohistochemical staining for α -smooth muscle actin (α -SMA), S-100, CD34, von Willebrand factor (factor VIII), vimentin, pan-cytokeratin, and Ki67 were also performed using serial sections. Table 1 provides details of the sources

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of the antibodies and conditions used for immunohistochemistry.

Histopathologically, the mass protruded into the lumen and was covered with intestinal epithelium, exhibiting an almost normal architecture (Fig. 1a). The lesion was located in the submucosa and was well-demarcated from the intestinal mucosa by thickened muscularis mucosa. The submucosal lesion consisted of loose connective tissue, smooth muscle fascicles, ganglion cells, and blood vessels of various sizes (Fig. 1b). The smooth muscle cells were arranged

irregularly throughout the lesion, being either solitary or present as bundles of cells with different sizes. In addition, there were many blood vessels of various sizes, including large vessels with irregularly thickened walls and hemangioma-like capillaries with dilated lumens. Moreover, mature ganglion cells with abundant eosinophilic cytoplasm surrounded by satellite cells were scattered in the loosely arranged nerve fibers (Fig. 1c). Although these components showed an irregular and disordered structure, there were no cellular atypia, increased mitotic figures, or invasive growth

Table 1. Antibodies Used for Immunohistochemistry

Antigen	Clonality (clone)	Host species	Dilution	Antigen retrieval	Supplier
α -SMA	Monoclonal (1A3)	Mouse	1:50	Autoclave	DAKO, Glostrup, Denmark
S-100	Polyclonal	Rabbit	Ready-to-use	None	DAKO
CD34	Monoclonal (EP373Y)	Rabbit	1:500	Autoclave	Abcam, Cambridge, UK
Factor VIII	Polyclonal	Rabbit	1:500	Autoclave	Abcam
Vimentin	Monoclonal (V9)	Mouse	1:500	Autoclave	Abcam
pan-Cytokeratin	Monoclonal (AE1/AE3+5D3)	Mouse	1:500	Autoclave	Abcam
Ki67	Polyclonal	Rabbit	1:1000	Autoclave	Abcam

SMA, smooth muscle actin; Factor VIII, von Willebrand factor.

Fig. 1. Histopathological findings for a submucosal mass located in the small intestine of a male F344 rat. (a) Whole-mount histopathology of the mass created by the image stitching function using an All-in-One Fluorescence Microscope BZ-X710 (Keyence, Osaka, Japan). A pedunculated mass covered with intestinal epithelium protruding into the lumen is evident. Hematoxylin and eosin (H&E) staining. (b) The submucosal lesion consists of loose connective tissue, smooth muscle fascicles (asterisks), and blood vessels of various sizes. H&E staining. Original magnification: 40 $\times$. (c) Scattered ganglion cells in the mass (arrowheads). H&E staining. Original magnification: 200 $\times$. (d) Toluidine blue staining. Note the infiltration of mast cells in the connective tissue of the mass. Original magnification: 400 $\times$.

to adjacent tissues. In the submucosal lesion, infiltration of inflammatory cells, including neutrophils, lymphocytes, plasma cells, and macrophages was rare, and degenerative and necrotic changes were not found. Toluidine blue staining revealed mild scattered infiltration of mast cells in the submucosa (Fig. 1d). No significant fibrosis nor formation of diverticula and cysts were observed. On immunohistochemical analysis, smooth muscle, ganglion, and endothelial cells were found positive for α -SMA and vimentin, S-100, and CD34 and factor VIII, respectively (Fig. 2a–e). Intestinal

epithelial cells lining the surface of the mass were diffusely positive for pan-cytokeratin and often for Ki67, particularly in the crypts, whereas components of the submucosal lesion were almost entirely negative for both the factors (Fig. 2f and g). Although the intestinal epithelium covering the surface of the mass was slightly taller than the adjacent normal area, Ki67-positive cells were located mainly in the crypts, and there were no abnormalities in the villous structures, including differentiation into goblet and Paneth cells. The results of the immunohistochemical analyses are sum-

Fig. 2. Immunohistochemical findings for a submucosal mass in the small intestine of a male F344 rat. (a) α -Smooth muscle actin. Irregularly arranged smooth muscle fibers and blood vessels are observed. Original magnification: 100 $\times$. (b) S-100. Scattered ganglion cells are observed. Original magnification: 200 $\times$. (c and d) CD34 and von Willebrand factor (factor VIII), respectively, in endothelial cells of blood vessels. Original magnification: 200 $\times$. (e) Vimentin is observed in blood vessels and interstitial fibroblasts. Original magnification: 200 $\times$. (f and g) pan-Cytokeratin and Ki67, respectively. Intestinal epithelial cells in the mucosa covering the mass are diffusely positive for pan-cytokeratin (inset in f) and often for Ki67 (inset in g), particularly in the crypts, whereas the components in the submucosal lesion are negative. Original magnification: 100 $\times$.

Table 2. Immunohistochemical Analysis of the Small Intestine Mass

	Mass			Surrounding normal tissues			
	Smooth muscles	Ganglion cells	Blood vessels	Smooth muscles	Ganglion cells	Blood vessels	Epithelial cells
α -SMA	+	—	+a	+	—	+a	—
S-100	—	+	—	—	+	—	—
CD34	—	—	+b	—	—	+b	—
Factor VIII	—	—	+b	—	—	+b	—
Vimentin	+/-	+/-	+/-a	+/-	+/-	+/-a	—
Cytokeratin	—	—	—	—	—	—	+
Ki67	—	—	—	—	—	—	+/-

a, positive in smooth muscle cells; b, positive in endothelial cells. —, negative; +/-, occasionally positive; +, diffusely positive. SMA, smooth muscle actin; Factor VIII, von Willebrand factor.

marized in Table 2.

Based on morphological findings, benign or malignant tumors originate from smooth muscles, peripheral nerves, and vascular tissues, and a hamartomatous lesion was considered for the differential diagnosis. The results of immunostaining for vimentin and pan-cytokeratin revealed that the components of the submucosal lesion were of mesenchymal rather than epithelial origin. The lesion consisted only of mature cells, and there was no evidence of cellular atypia, increased cell proliferation activity, or invasive growth to adjacent tissues, indicating that the mass was unlikely to be neoplastic tissue. Meanwhile, there was no scar formation, adhesion, or protrusion on the serosal surface, and none of the histopathological findings suggested remnants of the umbilical cord, such as significant fibrosis or formation of diverticula and cysts. Thus, the mass was considered to be a nonneoplastic hamartomatous lesion corresponding to NMVH in humans, which is characterized by irregular growth of mature tissues, including smooth muscle, ganglion cells, and vascular structures.

NMVH is considered a benign lesion with a nontumoral nature because surgical resection is curative, and there have been no documented cases of recurrence. However, patients with NMVH often present clinical signs, such as abdominal pain, obstructive symptoms, or gastrointestinal bleeding^{3, 5, 8}. Several cases of NMVH have shown clinical and histological findings similar to those of inflammatory bowel diseases, such as Crohn's disease and diaphragm disease, the latter being a rare gastrointestinal abnormality induced by prolonged intake of nonsteroidal anti-inflammatory drugs^{3, 9–11}. Thus, the pathological features of NMVH may represent a chronic form of Crohn's disease or diaphragm disease^{9, 12}. However, recent reports have suggested that NMVH may be a distinct entity because many cases are not associated with transmural chronic inflammation, granulomatous lesions, or prominent fibrosis, which are hallmarks of the abovementioned inflammatory bowel diseases^{4, 13}. In the current case, no clinical signs, such as blood in the feces or changes in body weight gain and food consumption, were observed throughout the experiment. In addition, there were no histopathological findings suggestive of chronic inflammation, granulomas, or fibrosis in the gastrointestinal tract. Although focal infiltration of mast cells was observed in the

present case, which is rare in human NMVH¹⁴, the extent of the infiltration was minimal and its relevance to pathogenesis was unclear. These findings supported that the current case is independent of inflammatory diseases and is a hamartomatous lesion corresponding to human NMVH.

Spontaneous hamartomas in the gastrointestinal tract of rodents are extremely rare. Most human NMVH cases have been protrusive, accompanied by clinical symptoms such as obstruction. Additionally, the single reported case in a dog also presented with mass-induced invagination of the cecum⁷. Likewise, the mass in the current case also protruded into the lumen, but did not cause erosions, ulcers, or obstructions, nor any clinical symptoms. Gastrointestinal lesions without obvious clinical or macroscopic findings are easily overlooked in routine toxicity studies. A recent report suggested that multiple factors may be involved in the pathogenesis of NMVH; in particular, ischemia may affect NMVH owing to the important roles of vascular damage in its development¹⁴. Further studies are needed to clarify the true frequency and mechanisms of NMVH in laboratory animals.

In summary, we describe a spontaneous case of hamartoma in the small intestine of a male F344 rat. Based on histopathological and immunohistochemical findings, the rat was diagnosed with NMVH of the small intestine. To the best of our knowledge, this is the first report of NMVH in rodents histologically resembling human cases.

Disclosure of Potential Conflicts of Interest: Takanori Yamada is an employee of Sanwa Kagaku Kenkyusho, Nagoya, Japan. The other authors declare no potential conflicts of interest.

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