

Case Report

Swim bladder tumors in the wavy medaka (*Oryzias latipes*)

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Abstract: Swim bladder tumors were detected in three out of 28 wavy medakas aged about 2 years old, all of which displayed abnormal swimming patterns caused by their spinal curvature. The tumors were located in the dorsal abdominal cavity. The swim bladder lumen was not detected in the region where it was originally assumed to be located, and that region was replaced with adipose tissue. The tumors were non-invasive, expansile, and encapsulated solid masses composed of a homogenous population of well-differentiated, densely packed, gas glandular epithelium-like cells. The tumor masses were connected to the rete mirabile, but the tumor cells did not infiltrate into them. Histopathologically, these tumors were diagnosed as adenomas originating from the gas glandular epithelium of the swim bladder. Spontaneous swim bladder tumors are rare in medaka, with an incidence of 0.02%; however, in the present study of wavy medaka, the incidence was much higher (10.7%). The long-term physical effects on the gas gland caused by swim bladder deformation considered to be a secondary effect of the spinal curvature may be an important factor in the proliferation of the gas glandular epithelium in the wavy medaka, resulting in the higher incidence of swim bladder tumors. (DOI: 10.1293/tox.2020-0058; J Toxicol Pathol 2021; 34: 107–111)

Key words: adenoma, gas gland, spontaneous, swim bladder, wavy medaka

The swim bladder in teleosts originates from an outgrowth of the anterior part of the alimentary canal and does not exist in mammals. It is the primary organ for controlling whole-body density, buoyancy, and sound production¹. Anatomically, the swim bladder wall comprises two layers: the tunica interna composed of a simple flat epithelium and the tunica externa composed of connective tissue^{2,3}. The volume of gas in the swim bladder is controlled by the action of both a rete mirabile and a gas gland. The rete mirabile is a dense bundle of parallel arterial and venous capillaries arranged side by side, and utilizes countercurrent blood flow within the net to act as a countercurrent exchanger. The gas gland comprises a folded cuboidal or columnar epithelium that secretes gas into the swim bladder. In toxicity and field studies, limited pathological lesions of the swim bladder have been reported⁴, because this organ is not routinely examined, although it is sometimes included by chance during sagittal or transverse whole-body sectioning of smaller fish⁵. Additionally, since the swim bladder is often punctured and deflated during pathological preparation, its lesions are commonly overlooked.

Spontaneous swim bladder tumors are rare in teleosts,

with only a few cases described in a handful of species⁶. The swim bladder tumors can be roughly classified into two types based on their origin: mesenchymal and epithelial tumors. The former originate from the smooth muscle and/or fibroblastic tissues of the swim bladder wall and are diagnosed as leiomyosarcomas⁷ and/or fibrosarcomas^{8,9}. These mesenchymal tumors occur in salmon and are frequently associated with a retroviral infection¹⁰. The latter originate from the swim bladder epithelium and are diagnosed as adenomas, papillary adenomas, and/or adenocarcinomas. Most spontaneous and chemical-induced swim bladder tumors in the teleosts are categorized as gas gland epithelial tumors. The epithelial tumors have been reported in medaka¹¹, mullet¹², guppy^{11,13}, cod¹⁴, seahorse¹⁵, and *Nothobranchius* fish¹⁶. In the present study, we encountered swim bladder tumors in three wavy medakas and described their detailed histopathological features.

Twenty-eight wavy medakas, aged about 2 years old, were sourced from the small stocks of wavy medaka at the Biological Research Laboratory, Nissan Chemical Corporation. These stocks were obtained via home breeding of some wavy medakas encountered naturally in the stocks maintained at the laboratory. The fish were maintained in dechlorinated tap water at $25 \pm 1^\circ\text{C}$ under a 16:8-hour light:dark photoperiod. The wavy medakas and one normal medaka were sacrificed by overexposure to CO_2 gas and fixed in Bouin's solution overnight, before being refixed in 10% neutral-buffered formalin. The fixed medakas were separated into two sections by mid-sagittal cut, and both sections were embedded in paraffin, sectioned at a thickness of $4 \mu\text{m}$, and stained routinely with hematoxylin and eosin for histopathological examination. This study was conducted according

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to the Guidelines for Animal Experimentation, Biological Research Laboratory, Nissan Chemical Corporation.

Histopathology of the Swim Bladder in the Normal and Wavy Medakas

The swim bladder in the normal medaka was located posterior and inferior to the head and body kidney, respectively, in the dorsal abdominal cavity that was divided by the diaphragm superior to the gastrointestinal tract. The shape of the swim bladders in sagittal section was a lateral prolate spheroid shape (Fig. 1a). The wavy medakas exhibited a spinal curvature characterized by dorsoventrally curved vertebrae, resulting in abnormal swimming patterns. The swim bladders in the wavy medakas were located in the dorsal abdominal cavity, same as in the normal medaka; however, they had a longitudinal oval shape in sagittal section (Fig. 1b). The gas gland and rete mirabile were located in similar positions in both the normal and wavy medakas at the cranial pole of the swim bladder. The gas gland was composed of three to four layers of pale eosinophilic vacuolated cuboidal epithelium (Fig. 1c and d) and was connected to the rete mirabile that had parallelly arranged blood capillaries (Fig. 1d).

Histopathology of Swim Bladder Tumors in the Wavy Medakas

The tumor of Fish No. 1 (male) was located posterior to the head kidney in the dorsal abdominal cavity (Fig. 2a). The other tumors of Fish No. 2 and 3 (female) were located inferior to the body kidney in the dorsal abdominal cavity and were connected to the rete mirabile (Fig. 3a and 4a). The rete mirabile of Fish No. 2 was slightly congested (Fig. 3b). In all three fish, the swim bladder lumen was not detected in the region where it was originally assumed to be located, and that region was replaced with adipose tissues (Fig. 2a, 3a and 4a). The tumor masses in these tissues were non-invasive, expansile, and encapsulated solid masses of proliferating tumor cells (Fig. 2b, 3b and 4b). No infiltration of tumor cells into the rete mirabile had occurred (Fig. 3c and 4b). The tumor masses were composed of a homogenous population of well-differentiated, densely packed, gas glandular epithelium-like cells. The tumor cells were arranged in cords, trabeculae, and solid patterns, supported by capillaries and minimal stroma. They exhibited various sizes and were of round to polygonal shape, with distinct cell borders and pale eosinophilic vacuolated cytoplasm (Fig. 2c). Multinucleate cells and cytomegalic cells were also scattered throughout. The nuclei exhibited anisonucleosis, with irregularly shaped and unclear nucleoli, although no mitotic figures were detected within the tumor masses. A few foci of adipocytes were scattered throughout the tumor (Fig. 4c). Based on these features, these tumors were diagnosed as adenomas originating from the gas glandular epithelium of the swim bladder. Additionally, these tumor cells did not seem to function as gas glandular epi-

thelium, since the swim bladder lumen did not form in these wavy medakas. With regards to histopathological lesions in other organs, there were large blood cysts in kidney and multiple hepatic cysts with necrosis and inflammation in Fish No.1, calcification in kidney and multiple hepatic cysts in Fish No. 2, and no lesions in Fish No. 3.

Swim bladder tumors can be induced in teleosts via exposure to environmental contaminants and carcinogens^{17, 18}. The chemical-induced swim bladder tumors are reported in the medaka exposed to 4-chloroaniline¹⁹, aniline¹⁹, N-

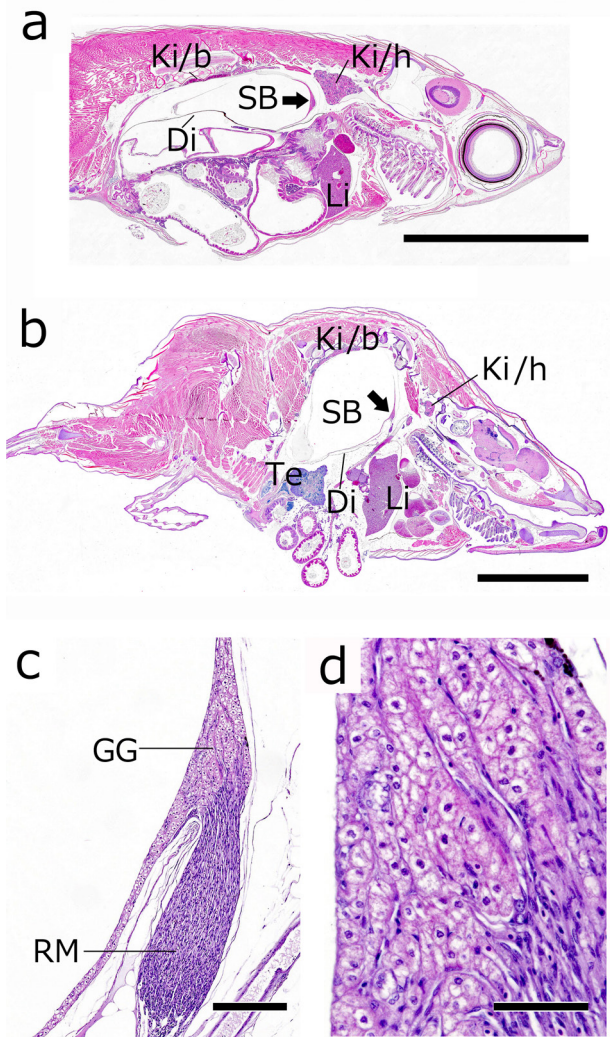


Fig. 1. Histopathology of the swim bladder in normal and wavy medakas. a) Loupe image of a sagittal section in normal medaka. Lateral prolate spheroid-shaped swim bladder located alongside the gas gland and rete mirabile (†). HE stain. Bar = 4,000 μ m. b) Loupe image of a sagittal section in wavy medaka. Longitudinal oval-shaped swim bladder (†). HE stain. Bar = 4,000 μ m. c) Low magnification of gas gland and rete mirabile in wavy medaka. HE stain. Bar = 200 μ m. d) High magnification of gas gland in wavy medaka. HE stain. Bar = 50 μ m. Di, diaphragm; GG, gas gland; Ki/b, body kidney; Ki/h, head kidney; Li, liver; RM, rete mirabile; SB, swim bladder; Te, testis

methyl-N²-nitro-N-nitrosoguanidine (MNNG)²⁰, or bis (tri-n-butyltin)oxide²¹; in the guppy exposed to methyl mercury chloride²²; and in the rainbow trout exposed to diethylnitrosamine¹⁷, methylazoxymethanol acetate¹⁷, benz(a)pyrene¹⁷, MNNG¹⁷, N-methylnitrosourea, dimethylbenz[a]anthracene¹⁷, or 2,6-dimethylintrosomorpholine²³. Conversely,

spontaneous swim bladder tumors are rare in teleosts, with an incidence of 0.02% (2/10,000) in medakas than 24 weeks of age and 0.14% (7/5,000) in guppies older than 13 weeks of age, which have been used in the control groups of a variety of carcinogenesis tests¹¹. In contrast, juvenile fish with skeletal deformations show a high prevalence of spontaneous

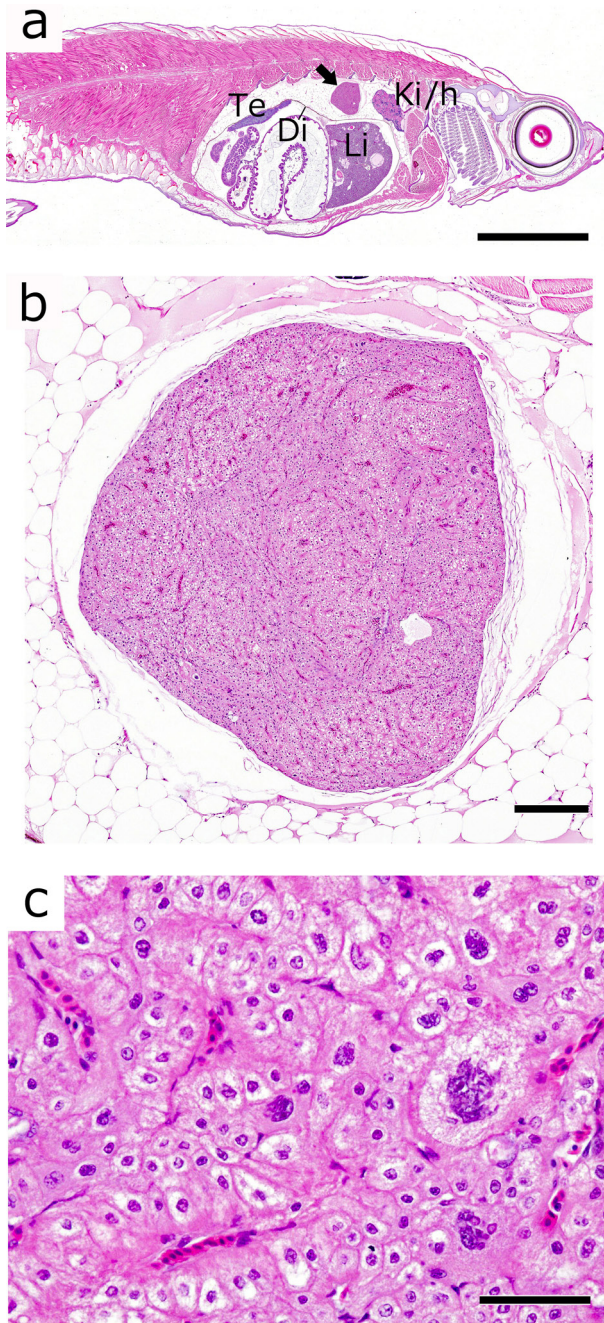


Fig. 2. Histopathology of gas gland adenoma in Fish No. 1. a) Loupe image of a sagittal section. Tumor mass (†). HE stain. Bar = 4,000 μ m. b) Low magnification of gas gland adenoma. HE stain. Bar = 200 μ m. c) High magnification of tumor cells. HE stain. Bar = 60 μ m. Di, diaphragm; Ki/h, head kidney; Li, liver; Te, testis.

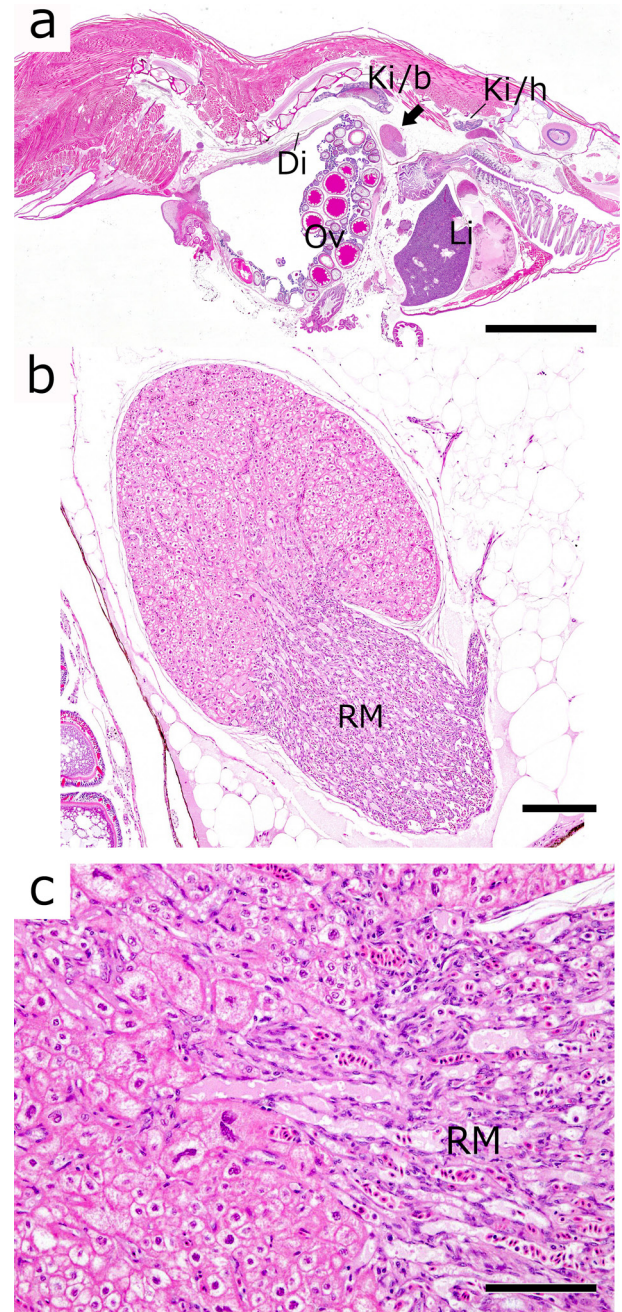


Fig. 3. Histopathology of gas gland adenoma in Fish No. 2. a) Loupe image of a sagittal section. Tumor mass and rete mirabile (†). HE stain. Bar = 4,000 μ m. b) Low magnification of gas gland adenoma and rete mirabile. HE stain. Bar = 200 μ m. c) Medium magnification of adenoma-rete mirabile junction. HE stain. Bar = 80 μ m. Di, diaphragm; Ki/b, body kidney; Ki/h, head kidney; Li, liver; Ov, ovary; RM, rete mirabile.

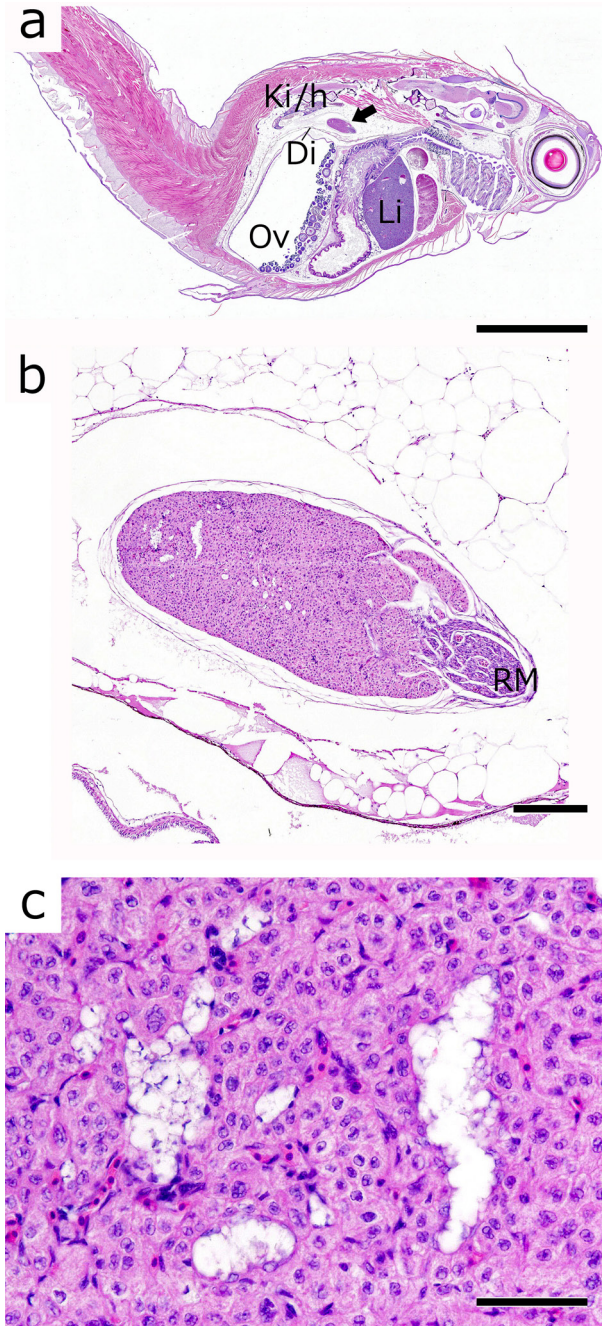


Fig. 4. Histopathology of gas gland adenoma in Fish No. 3. a) Loupe image of a sagittal section. Tumor mass and rete mirabile (\uparrow). HE stain. Bar = 4,000 μm . b) Low magnification of gas gland adenoma. HE stain. Bar = 200 μm . c) High magnification of tumor cells along with foci of adipocytes. HE stain. Bar = 40 μm . Di, diaphragm; Ki/h, head kidney; Li, liver; Ov, ovary; RM, rete mirabile.

proliferative changes in the swim bladder, including tumors. Such abnormalities have been found in Atlantic cod with notochord deformations¹⁴ and *Sparus aurata* with kypholordosis²⁴. Therefore, it has been suggested that the swim bladder tumors either induce severe skeletal deformations or are related to the genetic factors responsible for such de-

formations. In the present cases, the incidence of spontaneous swim bladder tumors was much higher at 10.7% (3/28) in the wavy medaka, compared with the normal variant. The wavy medaka develops due to a vertebral abnormality that is determined by an autosomal recessive gene (wavy; *wy*)²⁵ and is characterized by wavy dorso-ventral curves in the vertebral column^{26, 27}. There have been no reports of a relationship between the wavy gene and swim bladder tumors. In the present study, the swim bladder deformation was observed in wavy medakas, and this change was considered to be a secondary effect of the spinal curvature. Thus, the long-term physical effects of swim bladder deformation on the gas gland may be an important factor in the proliferation of the gas glandular epithelium in the wavy medaka, resulting in a higher incidence of swim bladder tumors. Furthermore, histological investigations of the gas glandular epithelium need to be conducted during the deformation process of the swim bladder in the wavy medaka.

Disclosure of Potential Conflicts of Interest: The authors declare that there is no conflict of interest.

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