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Case Report

Swim bladder tumor in the young adult scoliotic medaka (*Oryzias latipes*)

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Abstract: A swim bladder tumor was detected in one scoliotic medaka aged 22 weeks. The tumor was located in the dorsal abdominal cavity, with maximum dimension of 1,850 × 1,500 µm. No swim bladder lumen was identified, and the region where the swim bladder lumen would have been located, was replaced with adipose tissues. The tumor was a non-invasive, expansile, and encapsulated solid mass with a few cysts, and comprised a homogenous population of well-differentiated, densely packed, gas glandular epithelium-like cells. The tumor mass was connected to a rete mirabile that showed a hyperplastic capillary plexus; however, the tumor cells did not invade the rete mirabile, thereby revealing that the tumor was an adenoma originating from the gas glandular epithelium of the swim bladder. Since proliferative lesions in the swim bladder have been reported in some teleosts with skeletal deformations, including medaka, the occurrence of a spontaneous swim bladder tumor in teleosts is considered to be closely associated with various types of skeletal deformation, and spinal curvature in particular. (DOI: 10.1293/tox.2020-0088; J Toxicol Pathol 2021; 34: 157–160)

Key words: adenoma, gas gland, scoliosis, skeletal deformation, spontaneous, teleost

The swim bladder in teleosts is an organ that controls whole-body density, buoyancy, and sound production and originates from an outgrowth of the anterior part of the alimentary canal[1]. These swim bladders are classified into two types based on the presence or absence of a pneumatic duct, namely, a physostomous swim bladder (in Salmoniformes, Cypriniformes, Clupeiformes, etc.) or a physoclistous swim bladder (in Myctophiformes, Perciformes, Gadiformes, etc.). In teleosts with a physoclistous swim bladder and some kinds of the teleosts with a physostomous swim bladder, the filling and the emptying of the gas bladder are respectively made by a secretory section (gas gland and rete mirabile) and a resorbing section (oval body)[2, 3].

Spontaneous swim bladder tumors are rare in teleosts, with only few cases described in limited species[4]. These tumors have been reported in medaka[5], mullet[6], guppy[5, 7], cod[8], seahorse[9], and Nothobranchius fish[10]. The swim bladder tumors can be roughly classified into two types based on their origin: mesenchymal or gas gland epithelial tumors. Most swim bladder tumors are of the latter type and are diagnosed as adenomas, papillary adenomas, and/or adenocarcinomas. In our previous study, we reported swim bladder tumors in three wavy medakas[11], which were characterized by kypholordosis[12]. In the present study, we encountered a swim bladder tumor in a young adult scoliotic medaka, which was characterized by sideways curved vertebrae, and described the detailed histopathological features of this tumor.

Our one medaka, aged 22 weeks, was sourced from the stocks of medaka for fish toxicity studies at the Biological Research Laboratory, Nissan Chemical Corporation. The fish was maintained in dechlorinated tap water at 25 ± 1°C under a 16:8-hour light:dark photoperiod. The fish was sacrificed by overexposure to CO_2 gas and fixed in Bouin's solution overnight, before being refixed in 10% neutral-buffered formalin. Thereafter, the fixed fish was separated into two sections by mid-sagittal cut, and both sections were embedded in paraffin, serial sectioned at a thickness of 4 µm, and stained routinely with hematoxylin and eosin for histopathological examination. This study was conducted according to the Guidelines for Animal Experimentation, at the Biological Research Laboratory, Nissan Chemical Corporation.

The fish revealed growth abberations and decreased locomotor activity. It grossly presented scoliosis: the vertebra was laterally curved at the trunk region and the boundary between the trunk and tail regions (Fig. 1a). Histologically, the tumor was located posterior to the head kidney in the dorsal abdominal cavity that was divided by the diaphragm superior to the gastrointestinal tract, and compressed the diaphragm and head kidney in the left side section (Fig. 1b). The tumor mass had a maximum dimension of 1,850 × 1,500 µm. No swim bladder lumen was identified, and the region where the swim bladder lumen would have been located, was replaced with adipose tissues (Fig. 1b). Moreover, the

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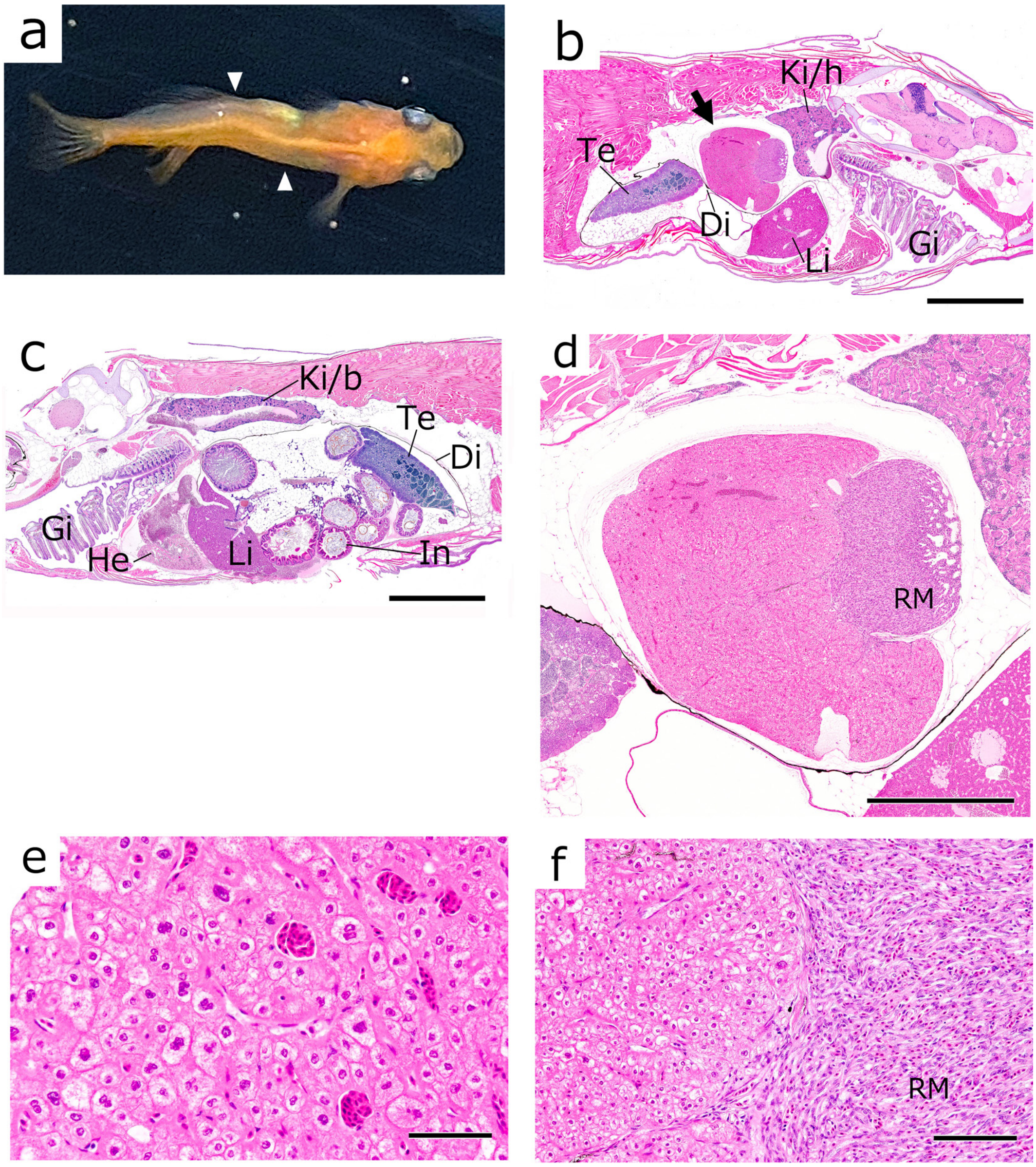

Fig. 1. a) Gross appearance of swimming scoliotic medaka. Vertebra showing laterally curved at trunk region (Δ) and boundary (Δ) between trunk and tail regions. b) Loupe image of a sagittal section (left side). Tumor mass (→) and rete mirabile. Tumor compressing diaphragm and head kidney without formation of swim bladder lumen. Hematoxylin and eosin (HE) stain. Bar = 2,000 µm. c) Loupe image of a sagittal section (right side). No tumor mass or swim bladder lumen. Thickening of body kidney. HE stain. Bar = 2,000 µm. d) Low magnification of gas gland adenoma and rete mirabile. Encapsulated expansile solid mass with a few cysts. Rete mirabile showing a hyperplastic capillary plexus. HE stain. Bar = 700 µm. e) High magnification of tumor cells. Tumor mass supported by capillaries and minimal stroma. Homogenous population of well-differentiated, densely packed, gas glandular epithelium-like cells with pale eosinophilic vacuolated cytoplasm. HE stain. Bar = 45 µm. f) Medium magnification of boundary between gas gland adenoma and rete mirabile. No infiltration of tumor cells into rete mirabile. HE stain. Bar = 90 µm. Di, diaphragm; Gi, gill; He, heart; Ki/b, body kidney; Ki/h, head kidney; In, intestine; Li, liver; RM, rete mirabile; Te, testis.

tumor mass was unevenly located on the left side of abdominal cavity, and no mass was detected in the right side sections (Fig. 1c). Furthermore, the body kidney showed thickening caused by the loss of swim bladder lumen. The tumor was a non-invasive, expansile, and encapsulated solid mass with a few cysts, which contained an eosinophilic substance (Fig. 1d), and comprised a homogenous population of well-differentiated, densely packed, gas glandular epithelium-like cells. Histologically, the tumor cells were arranged in cords, trabeculae, and solid patterns, supported by capillaries and minimal stroma. They were of various sizes and of round to polygonal shape with distinct cell borders and pale eosinophilic vacuolated cytoplasm (Fig. 1e). Anisonucleosis was observed with irregularly shaped and indistinct nucleoli, although no mitotic figures were detected. The tumor mass was connected to a rete mirabile that showed a hyperplastic capillary plexus with focal dilated lumen (Fig. 1d); however, the tumor cells did not invade the rete mirabile (Fig. 1f). From these histological features, this tumor was diagnosed as an adenoma originating from the gas glandular epithelium of the swim bladder. Notably, histopathological lesions in other organs were observed, such as multiple cysts in the liver.

The morphology of swim bladder tumor in the present case was similar to that in the wavy medakas, described in our previous report[11]; however, the rete mirabile was enlarged by the hyperplastic capillary plexus in this case. The proliferated rete mirabile in the swim bladder tumors was also detected in Sparus aurata[13]. Although it was unclear whether this lesion was a specific change in the swim bladder tumors, the capillary plexus connected to the tumor seemed to be more developed, resulting from the large tumor mass in the present case.

Swim bladder tumors can be induced in teleosts via exposure to environmental contaminants and carcinogens[14, 15]. Chemically-induced swim bladder tumors originating from gas glandular epithelium have been reported in the medaka exposed to N-methyl-N'-nitro-N-nitrosoguanidine[16], 4-chloroaniline[17], aniline[17], or bis(tri-n-butyltin)oxide[18] and in the guppy exposed to methyl mercury chloride[19]. Spontaneous swim bladder tumors are rare, with an incidence of 0.02% (2/10,000) in medakas older than 24 weeks of age and 0.14% (7/5,000) in guppies older than 13 weeks of age that have been used in the control groups for various carcinogenicity tests[5]. In contrast, the incidence of swim bladder tumors is markedly higher at 10.7% (3/28) in wavy medakas[11]. Additionally, it is reported that the juvenile fishes with skeletal deformations exhibit a high prevalence of spontaneous proliferative changes in the swim bladder, including the swim bladder tumors. Such abnormalities have been found in *Sparus aurata*[13] with kypholordosis and Atlantic cod[8] with notochord deformations and subsequent axial skeleton deformation. Because a swim bladder tumor was additionally detected in a scoliotic medaka in the present study, these findings collectively support the occurrence of spontaneous swim bladder tumors is closely associated with various types of skeletal deformation, and spinal curvature in particular. Moreover, it has been proposed that failure to gulp surface air by few farm-raised juvenile marine fishes leads to an abnormally closed swim bladder and spinal curvature[20]. In our previous and present studies, all spinally curved medakas with a swim bladder tumor had an abnormally closed swim bladder lumen. In addition, our wavy medakas without a swim bladder tumor showed swim bladder deformation induced as a secondary effect of spinal curvature. Therefore, the swim bladder tumors in the wavy or scoliotic, medaka is likely involved with effects caused by an uninflated swim bladder or by swim bladder deformation, on the proliferation of gas glandular epithelium.

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

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