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**Case Report**

# Spontaneous seminoma in medaka (*Oryzias latipes*)

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**Abstract:** Although spontaneous development of seminoma is rare in medaka, we encountered spontaneous testicular tumors located within the abdominal cavity in two adult medakas. The growth patterns of the tumors were a combination of solid and cord arrangements in one of the two cases (Case I) and lobular in the other case (Case II). The tumor cells resembled the cells at different stages of spermatogenesis, and a small number of oocyte-like cells were also scattered within the tumor. The tumor with solid and cord patterns showed loss of normal testicular architecture, and the tumor cells had partly invaded the dorsal muscular tissue and metastasized to the liver, kidney, and eye. The tumor with a lobular pattern did not exhibit local invasion or metastasis. The tumors were diagnosed as seminomas based on their histopathological characteristics, and the tumor in Case I was observed to be more malignant than that in Case II. (DOI: 10.1293/tox.2021-0024; J Toxicol Pathol 2022; 35: 95–98)

**Key words:** seminoma, malignant, germ cell, testis, medaka, fish

Germ cell tumors in fishes, seminoma and dysgerminoma, have been reported in medaka, zebrafish, fathead minnow, and other freshwater fishes[1–5]. In zebrafish, spontaneous seminomas have been relatively known, and chemically-induced seminomas have been reported following N-ethyl-N-nitrosourea, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), or 7,12-dimethylbenz[a]anthracene (DMBA) treatments[6, 7]. Conversely, in medaka (*Oryzias latipes*), spontaneous tumor development in organs other than the liver, including testis, is rare[8], and the frequency of germ cell tumors in previous reports was 0% (0 out of 961 males and females in life-span studies on spontaneous tumor development)[9] and 0.26% (14 males and 12 females, including chemically exposed medakas, out of approximately 10,000 males and females in carcinogenesis studies)[2]. We encountered spontaneous testicular tumors in two adult medakas (Cases I and II). This report describes the histopathological findings of these tumors.

The two medakas (*Oryzias latipes*) examined in this report were not chemically treated and were obtained from the breeding stock that had been maintained at the Biological Research Laboratories, Nissan Chemical Corporation. These medakas were judged to be male, due to the shape of the fins. The medakas were maintained in dechlorinated tap water at 25 ± 2°C and fed with Artemia once a day. The photoperiod of the light/dark cycle was 16 h/8 h. The medakas were euthanized using $CO_2$ gas and fixed overnight in Bouin's fluid before being re-fixed in neutral buffered formalin, embedded in paraffin, cut into 4 µm thick sagittal sections, and stained with hematoxylin-eosin (HE). This study was conducted in accordance with the Guidelines for Animal Experimentation, Biological Research Laboratory, Nissan Chemical Corporation.

The clinical data and the macropathological and histopathological findings are described below. The medaka in Case I was a "wavy medaka" with congenital spinal curvature characterized by a dorsoventrally curved vertebrae[10], and aged about 12 months when the tumor was found. Macroscopically, the abdomen was considerably distended by the tumor mass (Fig. 1). Histologically, the tumor mass (size, 13 mm) filled the abdominal cavity and had displaced the abdominal organs (Fig. 2). The tumor cells were arranged in solid and cord patterns and resembled spermatogonia, spermatocytes, and spermatids (Fig. 3A and 3B). The spermatogonia-like cells had round nuclei with prominent nucleoli and moderate amounts of cytoplasm. The spermatocyte-like cells were smaller than the spermatogonia-like cells and had comparatively dense nuclei, while the spermatid-like cells were the smallest neoplastic cells with dense nuclei and scant cytoplasm. Mitotic figures were rarely observed in the spermatogonia- and spermatid-like cells; however, the spermatocyte-like cells often exhibited morphology similar to the meiosis phases. Accumulation of mature spermatozoa-like cells was not observed in the tumor. Additionally,

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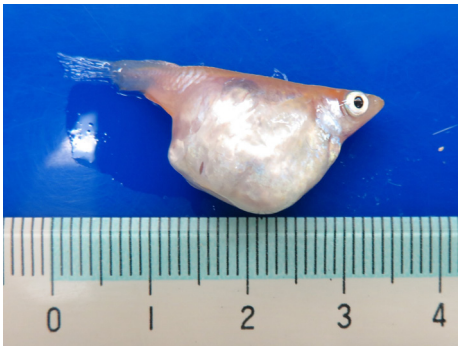


**Fig. 1.** Case I: Macroscopic appearance of the wavy medaka. The abdomen was considerably distended.

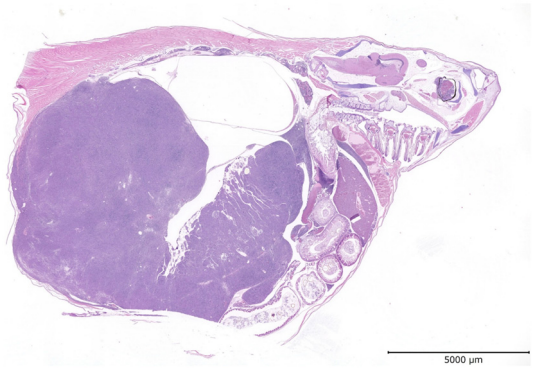


**Fig. 2.** Case I: Loupe image of the tumor. The tumor mass filled the abdominal cavity. Hematoxylin-eosin (HE) stain. Bar=5,000 µm.

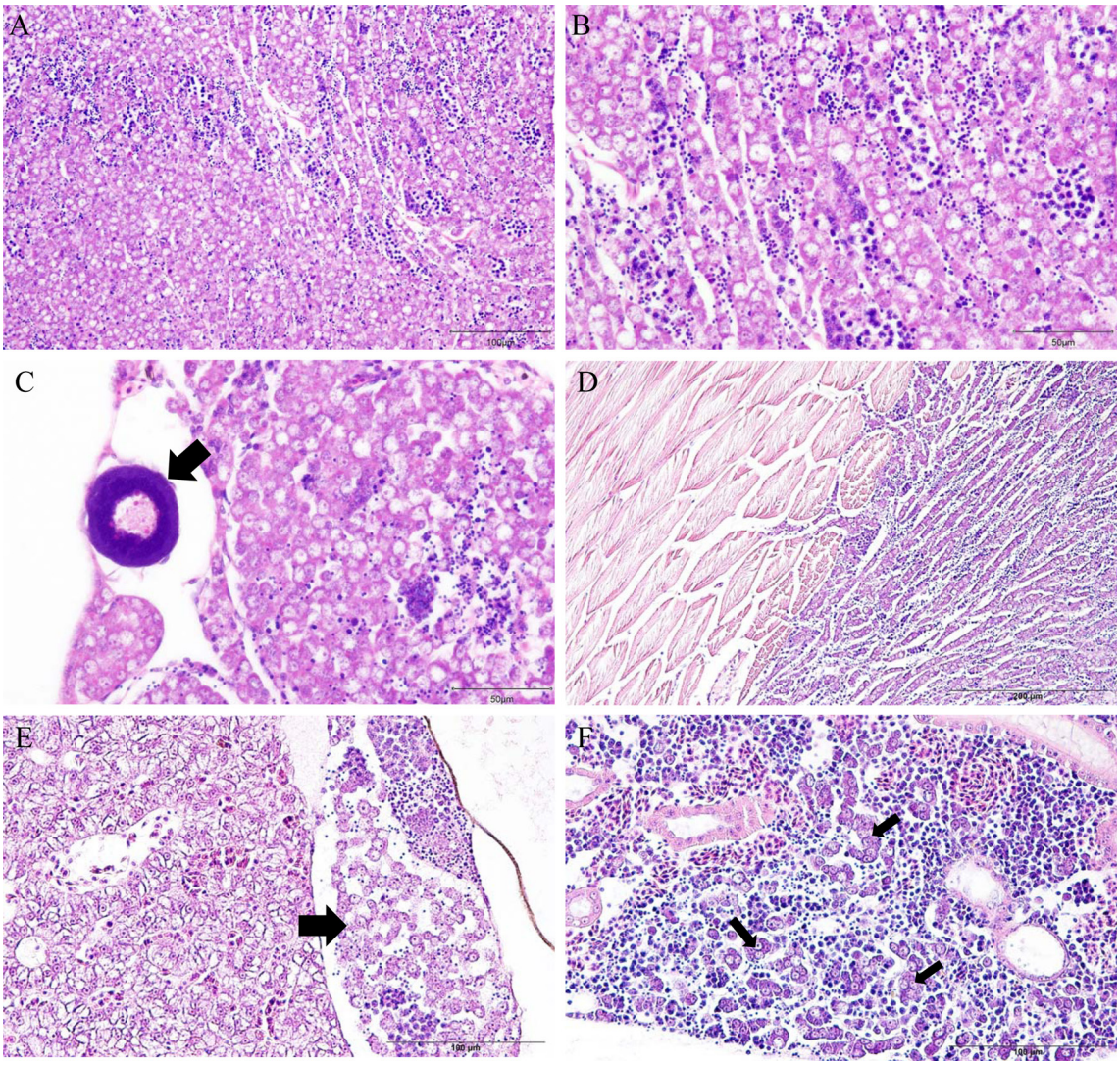


**Fig. 3.** Case I: Histological images of the tumor. Hematoxylin-eosin (HE) stain (A–F). (A) The tumor cells were arranged in solid and cord patterns. Bar=100 µm. (B) The tumor cells resembling spermatogonia, spermatocytes, and spermatids proliferated and exhibited different stages of spermatogenesis. Bar=50 µm. (C) The oocyte-like cell with a large vesicular nucleus and dense basophilic cytoplasm was observed in the tumor (black arrow). Bar=50 µm. (D) The tumor cells invaded the muscular tissue. Bar = 200 µm. (E) Metastasis of the tumor cells was observed in the liver (black arrow). Bar=100 µm. (F) Metastasis of the tumor cells was observed in the kidney (black arrow). Bar=100 µm.

a small number of large cells resembling oocytes were scattered within the tumor (Fig. 3C). The oocyte-like cells had characteristics of perinucleolar oocytes with large vesicular nuclei and dense basophilic cytoplasm. The tumor had partly invaded the dorsal muscular tissue (Fig. 3D), sparing the swim bladder. The tumor cells showed metastasis to the liver, kidney, and eye (Fig. 3E and 3F).

In Case II, the medaka was male, aged about nine months, and belonged to the control group of a 3-day toxicity study. No abnormal clinical signs were observed during the study period. The tumor mass was not detected on macroscopic examination. Histologically, the tumor mass (size, 3 mm) was confined within the abdominal cavity and was continuous with the efferent duct (Fig. 4). The tumor did not exhibit local invasion or metastasis to other organs. The tumor cells were arranged in a lobular pattern with a fibrous stroma (Fig. 5A). As in Case I, this tumor was also composed of three types of neoplastic germ cells; however, spermatocyst-like structures comprising the same type of tumor cells (spermatogonia-, spermatocyte-, or spermatid-like cells) were characteristically formed in this tumor (Fig. 5B). Spermatocyst, in normal testis, is the functional unit composed of a clonal group of spermatogenic cells that are surrounded by the cytoplasmic arm of a Sertoli cell. Degenerating cells were often observed, and there were a few oocyte-like cells within the tumor.

In previous reports of seminomas in fishes (medaka, zebrafish, common carp, and crucian carp), the principal cellular patterns of the neoplasms are solid, lobular, or a combination of these, and the tumor cells in solid tumors are often arranged in a cord-like pattern[1, 2, 4, 5]. The components of fish seminomas are similar to the cellular stages of spermatogenesis, namely, spermatogonia, spermatocytes, and spermatids, and the developmental progression toward more mature cell types is detected in these cases[1, 2, 4, 5]. In addition, the proliferation of primitive spermatogonia-like cells with loss of spermatocytic differentiation has been reported in DMBA- or MNNG-induced seminoma in zebrafish[11]. In the present cases, the striking histological findings were solid or lobular proliferation of the tumor cells resembling the male germ cells observed in different stages of spermatogenesis, and these findings were similar to those of seminoma described in previous reports. Therefore, the tumors were diagnosed as seminomas. Although a few oocyte-like cells (testis-ova) were also scattered within the tumors, it is known that the appearance of testis-ova is common in seminomas of medaka[2]. Germ cells in fishes have sexual bipotentiality, and spontaneous development and chemical induction of testis-ova in normal testis have also been reported as non-neoplastic lesions in various fishes, including medaka[1, 12–14].

To our knowledge, there have been no reports on the distinction between benign and malignant seminomas in fishes, including medaka, and the diagnostic criteria for malignancy are unknown. When the two seminomas in the present report were compared, the tumor in Case I showed loss of normal testicular architecture with a solid and cord proliferation pattern, and metastasis and invasion of the tumor cells. Conversely, metastasis and invasion of the tumor cells were not observed in the tumor of Case II. Therefore, the tumor in Case I seemed to be more malignant than the tumor in Case II. The invasion and metastasis of neoplastic

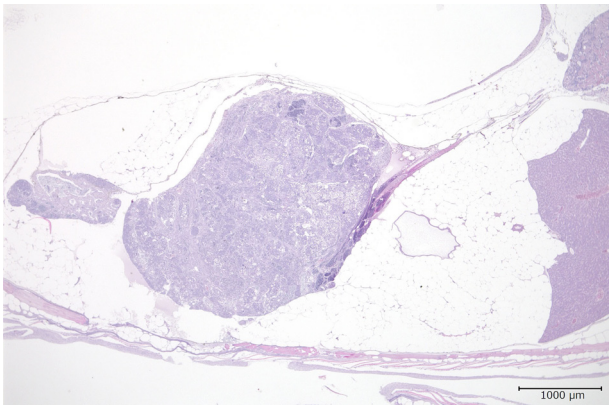


**Fig. 4.** Case II: Loupe image of the tumor. The tumor mass was found in the abdominal cavity. Hematoxylin-eosin (HE) stain. Bar=1,000 µm.

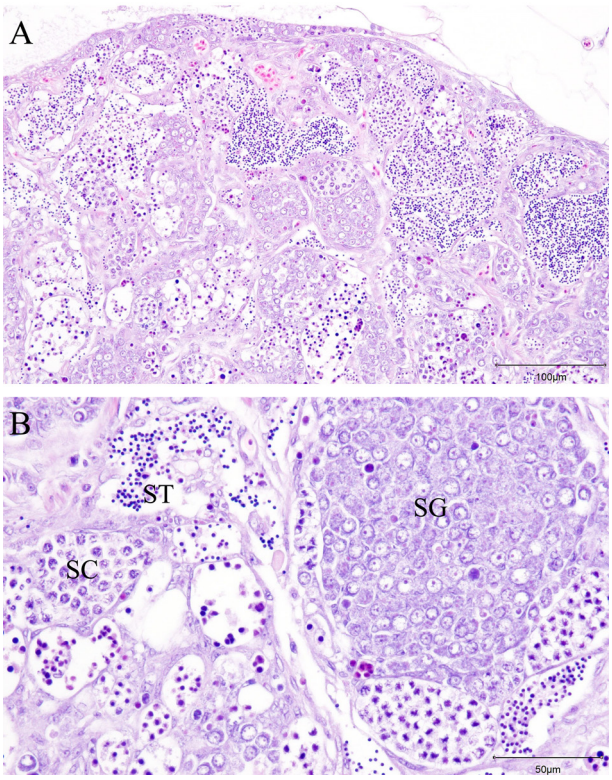


**Fig. 5.** Case II: Histological images of the tumor. Hematoxylin-eosin (HE) stain (A and B). (A) The tumor cells were arranged in a lobular pattern. Bar=100 µm. (B) Spermatocyst-like structures comprising the same type of the tumor cells were observed. SG, Spermatogonia-like cells; SC, Spermatocyte-like cells; ST, Spermatid-like cells. Bar 50 µm.

cells to other organs in seminomas of medaka and common carp[2, 5], such as in the present Case I, has been previously reported. On the other hand, metastasis of tumor cells to distant organs is uncommon in rodent seminomas[15]. Invasion of tumor cells throughout the testicular parenchyma has been reported in malignant seminomas of rats[16–18], although the tumor was confined within the tunica albuginea. There was a description that metastasis of canine seminomas occurs only in a small percentage of clinical cases[19]. Compared to rodents and dogs, metastasis of seminomas may be relatively common in fishes, such as medaka, because the testis is not demarcated by a thick capsule.

In Case I, the medaka showed dorso-ventral curvature of its vertebral column, the so-called wavy medaka. In previous reports, the occurrence of a spontaneous swim bladder tumor in fish is considered to be closely associated with various types of skeletal deformation, and wavy medaka in particular[20, 21]. However, there have been no previous reports of seminoma in wavy medaka. Furthermore, in the present report, the relationship between the development of the seminoma and skeletal deformation remains unclear.

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

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