

A Histopathological Study on Nephroblastomas of the Japanese Eel

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Renal tumors occurred at the incidence of 0.2-0.3% in the population of 2 year old Japanese eel *Anguilla japonica* (100-300 g in body weight) in an eel-farm in 1985. Diseased fish externally displayed swelling of the region of posterior kidney and the atrophic body. The posterior kidneys included neoplasm and were markedly swollen from 7 to 30 times as large as a normal kidney, and tumor nodules pressed the normal tissues of kidney. Epithelial cells, mesenchymal spindle cells, smooth muscle cells, striated muscle cells, hyaline cartilage cells, fibrocytes and collagenous fibrous tissue were found as components of the neoplasm. Tumors were classified according to elements into adenosarcoma, adeno-spindle-cell sarcoma, adeno-striated muscle-cell sarcoma, and mixed type. These histopathological features indicated that renal tumors were nephroblastoma (Wilms tumor). In some diseased fish, renal tumor cells metastasized into livers.

Key words : Eel. Nephroblastoma. Metastasis.

Nephroblastoma have been occasionally found in cultured rainbow trout *Salmo gairdneri* (HARSHBARGER 1972, 1974, 1976, ODENS *et al.* 1973, MIZUHASHI *et al.* 1979) and other salmonids (HARSHBARGER 1974, 1975, 1976), striped bass *Morone saxatilis* (HELMBOLDT and WYLAND 1971, HARSHBARGER 1972, 1976), crucian carp *Carassius carassius* (HARSHBARGER 1976), banded cichlid *Cichlasoma severum* (HARSHBARGER 1977), koi carp *Cyprinus carpio* (HARSHBARGER 1987) and rose bitterling *Rhodeus ocellatus* (HARSHBARGER 1978). This neoplasm had happened in Japanese eel *Anguilla japonica* cultured in Taiwan (EGUSA personal communication). Nephroblastoma occurred at the incidence of 0.2-0.3% in the population of pond-raised Japanese eel in an eel-farm in Mie Prefecture, in November in 1985. This study confirmed the histopathological features of eel nephroblastomas.

Materials and Methods

20 tumor-laden eels and 6 normal eels were collected in an eel-farm in November in 1985. 13 tumor-laden eels were provided for weighing the tumor-laden kidney, internal observation and histopathological study. 7 other fish were kept in an aquarium at 20 °C water temperature for about one month and the mortality was investigated. 6 normal fish were provided for weighing the kidney. Tumor-laden kidney and visceral organs were fixed with 10 % formalin solution and Bouin's fluid, and the thin sections were stained with Hematoxylin-eosin (H-E), PAS, Azan, PTAH, silver stain and Toluidin blue.

Results

External and dissected views

Each fish exhibited extreme expansion of the region of posterior kidney, and hemorrhage and ulceration in the surface of lesion. Their trunks and tails became thin. The kidneys included neoplastic nodules and were markedly expanded as big as the size of a hen's egg (Figure 1). Tumor-laden kidney body ratios were from 4.0 to 18 % and the average of 13 fish was 11.4 %. On the other hand, kidney body ratios of 6 normal fish were from 0.4 to 0.7 % and the average was 0.6 % (Table 1). The livers of 3 diseased fish showed small white nodules in the inside. Stomachs and intestines of all fish had no food matter.

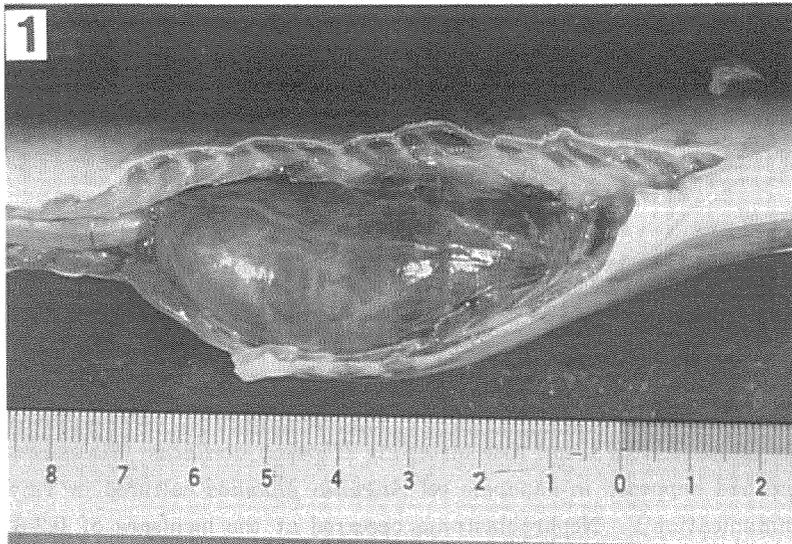


Figure 1. A dissected view of posterior kidney including nephroblastoma. The tumor-laden kidney enlarged as large as a hen's egg.

Among 7 tumor-laden fish held in an aquarium, 5 fish survived for one month except for two dead fish which died due to fungal infections.

Table 1. Sizes of nephroblastomas in the Japanese eel

Fish	Body weight	Tumor-laden fish		Body weight	Control fish	
		Kidney weight	Kidney /Body		Kidney weight	Kidney /Body
1	300 g	40 g	13.3%	115 g	0.5 g	0.4%
2	180	25	13.9	150	1.0	0.7
3	105	19	18.1	100	0.4	0.4
4	290	30	10.3	115	0.7	0.6
5	215	36	16.7	128	0.8	0.6
6	180	18	10.0	125	0.7	0.6
7	250	10	4.0			
8	180	20	14.4			
9	253	23	8.9			
10	215	13	5.8			
11	180	20	11.1			
12	165	18	10.6			
13	190	20	10.5			
Mean			11.4			0.6
± S.E.			4.0			0.1

Histopathological features

On histopathological observation, epithelial cells, mesenchymal spindle cells, smooth muscle cells, striated muscle cells, hyaline cartilage cells, fibrocytes and collagenous fibrous tissues were found as the components of renal tumors. Renal tumors were classified according to the dominant neoplastic elements into the following types.

1. Adenosarcoma

Three fish had this type of tumor. Tumor nodules were mostly composed of various sizes of masses of epithelial cells and separated by collagenous fibrous tissues including small numbers of spindle cells and striated muscle cells (**Plate I - 1**). Epithelial cell masses were composed of variously differentiated cells. The well differentiated epithelial cells displayed ciliation, columnar shapes and the acidophilic granular cytoplasm forming blind tubules on the basement membranes accompanying blood running capillaries (**Plate I - 2**). These tubules occasionally included PAS positive matter in the lumens. Tubule forming epithelial cells were occasionally vacuolized, necrotized and then calcified. Moderately differentiated epithelial cells were columnar or cuboidal, poorly ciliated or unciliated, and had weak basophilic or acidophilic cytoplasm. These epithelial layers were complexly folded or partially formed

abortive tubules. The folded epithelial layers showed the unclear relationship to the cells of their basement membrane (**Plate I-3**). Capillaries were poorly formed between the basement membranes of the cells forming abortive tubules. On the other hand, poorly differentiated epithelial cells exhibited spindle shapes and many mitotic figures. These cells had proliferated so complexly that the basement membranes and capillaries could not develop (**Plate I-3**). Abortive glomeruli proliferated in the periphery of epithelial cell masses. Most abortive glomeruli were primitive and only formed budding or papillary projections of round or cuboidal, basophilic cells and the underlying fibrous matter into the spaces out of the capsules which consisted of layers of basophilic cuboidal cells and the basement membrane (**Plate I-4**). No projections formed the pattern of capillary. These epithelial cell masses were separated by fibrous connective tissues in which spindle cells, striated muscle cells and pigment-laden macrophages were embedded. The central areas of tumor nodules occasionally had been necrotized and were either calcified or replaced by loose fibrous tissues. The periphery of nodules were infiltrative and invasive into kidney tissues, in which there were proliferation of small masses of epithelial cells.

2. Adeno-spindle cell-sarcoma

Six fish had this type of tumor. Tumor nodules were mostly composed of spindle shaped cells and masses of variously differentiated epithelial cells and abortive glomeruli (**Plate II-1**). These spindle cells displayed mitotic figures and densely or loosely formed the interwaving and interlacing pattern. Most spindle cells resembled poorly differentiated epithelial cells and had no fibrous stroma in them (**Plate II-2**). Spindle cells partially differentiated into primitive smooth muscle cells (**Plate II-3**) accompanying the mesh-works of argyrophil fibers (**Plate II-4**), primitive striated muscle cells, fibrous cells producing collagenous matter (**Plate III-1**), and capillary forming cells accompanying with/without blood circulation. Hyaline cartilage cells also proliferated among spindle cells (**Plate III-2**). Central areas of spindle cell masses became edematous and hemorrhagic. The neoplasms were invasive into the surrounding kidney tissues, in which small masses of spindle cells and epithelial cells proliferated (**Plate III-3**).

3. Adeno-striated muscle cell-sarcoma

This type of tumor was found in two fish. Striated muscle cells, varying from primitive to well differentiated, were the dominant element of the neoplasms (**Plate III-4, IV-1**). Masses of the striated muscle cells were separated by collagenous fibers. Among the muscle cell masses, epithelial cells, abortive glomeruli and spindle cells were embedded. The primitive muscle cells exhibited short columnar shapes, the thin myofibrils, the much sarcoplasm, the centralizing nuclei and the unclear endomysium. Their myofibrils were so primitive that the striation of myofibrils was partially either clear or unclear. The moderately differentiated

muscle cells showed elongated columnar shapes, the clearly striated thick myofibrils, the lesser sarcoplasm, the centralizing and marginal nuclei, and the endomysium of argyrophil fibers. Neoplasms were invasive into the surrounding kidney tissues.

4. Mixed type

This type of tumor was found in two fish. The tumor was composed of individually rising neoplastic nodules. Some nodules were adenosarcomas and adeno-spindle cell-sarcomas, and the others were adeno-striated muscle cell-sarcomas. All neoplastic nodules were invasive.

5. Metastasis

The livers of two fish having adeno-spindle cell-sarcoma exhibited metastatic lesions. Smaller metastatic lesions included spindle cells displaying mitosis and placed in veins and the surrounding parenchyma (**Plate IV- 2**). Large lesions extending into to parenchyma contained spindle cells, fibrocytes, epithelial cells forming tubules, poorly differentiated epithelial cells and abortive glomeruli (**Plate IV-3**). The liver of one fish having adeno-striated muscle cell-sarcoma showed metastatic lesions of spindle cells and striated muscle cells in veins and the surrounding parenchyma (**Plate IV-4**). The livers of all fish showed from slight to moderate infiltration of macrophages around the veins. Hepatic cells did not show obvious changes. No metastatic lesion and no obvious change was found in other visceral organs.

Discussion

Eel renal tumors were composed of various types of neoplastic cells such as epithelial cells, mesenchymal spindle cells, striated muscle cells, smooth muscle cells, hyaline cartilage cells, fibrocytes and collagenous fibrous tissues. These neoplastic cells showed various degrees of differentiation. The features of neoplasms were varied such as adenosarcoma, adeno-spindle cell-sarcoma, adeno-striated muscle cell-sarcoma. These characteristics indicated that renal tumors were nephroblastoma and Wilms tumor (ROBBINS 1967) and very much resembled those found in rainbow trout (HARSHBARGER 1972, ODENS *et al.* 1973, MIZUHASHI *et al.* 1979) and striped bass (HELMBOLDT *et al.* 1971, HARSHBARGER 1972). The carcinogens have been unknown in these spontaneous cases. ASHLEY (1970) reported rainbow trout fed experimentally Dimethylnitrosamine (DMN) for 20 months, exhibiting nephroblastomas. KIMURA (1976) also showed that rainbow trout fed MNNG, formed nephroblastomas. These results on feeding experiments indicated that feeding on carcinogens caused nephroblastomas. On the other hand, MIZUHASHI (1978) reported that the injection with a high level of DMN (5.6 ml/kg B.W.) and MNNG (10.0 mg/kg B. W.) resulted in failure to cause nephroblastoma in the Japanese eel.

In this study, nephroblastomas were found to occur at the considerable incidence in the

pond-raised Japanese eel. This indicated that some carcinogens would cause this tumor. It is highly speculative whether this tumor resulted from chemical carcinogens present in the eel diets or the pond water, or carcinogenic virus. Viruses were isolated from eel nephroblastomas but the particular virus is still unclear (SANO personal communication). What virus or chemical carcinogens would induce eel nephroblastomas will be examined in the further study.

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Explanation of Plate I

- Fig. 1. Adenosarcoma type of nephroblastoma. Neoplastic nodules were composed of epithelial cells and abortive glomeruli, and separated by collagenous fibrous tissue. Azan, X50.
- Fig. 2. A detail of well-differentiated epithelial cells displaying ciliation, columnar shapes, acidophilic granular cytoplasm and tubular formation on the basement membranes accompanying capillaries. Azan, X160.
- Fig. 3. A detail of moderately and poorly differentiated epithelial cells. The moderately differentiated cells formed the folded epithelial layers (upper half of nodule). The poorly differentiated cells showed spindle shapes, complex proliferation and mitotic figures. Azan, X160.
- Fig. 4. Abortive glomeruli showing budding and papillary projections of basophilic cuboidal cells and the underlying fibrous matter into the spaces from the capsules. Azan, X160.

Explanation of Plate II

- Fig. 1. Adeno-spindle cell-sarcoma. Spindle shaped cells dominantly proliferated and epithelial cells forming tubules (E) and abortive glomeruli (G) were embedded among them. Azan, X50.
- Fig. 2. A detail of spindle cells. They proliferated displaying the interlacing pattern and many mitotic figures. Azan, X320.
- Fig. 3. A detail of smooth muscle cells in the nodule of adeno-spindle cell-sarcoma. They proliferated exhibiting the interlacing pattern. PTAH, X320.
- Fig. 4. A detail of mesh-work stroma of smooth muscle cells. The meshwork stroma consisted of argyrophil fibers. Silver stain, X160.

Explanation of Plate III

- Fig. 1. A detail of fibrocytes producing collagenous fibrous matter (coloring gray) and capillary forming cells (arrows) in the nodule of adeno-spindle cell-sarcoma. Azan, X320.
- Fig. 2. A detail of hyaline cartilage cells (C) in the nodule of adeno-spindle cell-sarcoma. PAS, X160.
- Fig. 3. A detail of primitive neoplastic nodules rising in the kidney tissues surrounding nodules of adeno-spindle cell-sarcoma. These primitive nodules were composed of basophilic spindle cells. Azan, X160.
- Fig. 4. Adeno-striated muscle cell-sarcoma. Striated muscle cells dominantly proliferated and their masses were separated by fibrous connective tissue. Azan, X50.

Explanation of Plate IV

- Fig. 1. A detail of striated muscle cells. The moderately differentiated muscle cells included thick myofibrils. The primitive muscle cells included thin myofibrils and centralized nuclei. Azan, X100.
- Fig. 2. Metastatic lesion in the liver of fish having adeno-spindle cell-sarcoma type of nephroblastoma. Epithelial cells and spindle cells proliferated in hepatic parenchyma. H-E, X160.
- Fig. 3. Metastatic lesion in the liver of fish having adeno-spindle cell-sarcoma type of nephroblastoma. Epithelial cells, abortive glomeruli (G) and spindle cells proliferated penetrating into the surrounding hepatic tissue. H-E, X160.
- Fig. 4. Metastatic lesion in the liver of fish having adeno-striated muscle cell-sarcoma type of nephroblastoma. Primitive striated muscle cells invaded the hepatic tissues. H-E, X200.

