Nephroblastomas in the Japanese Eel, *Anguilla japonica* Temminck and Schlegel

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**ABSTRACT**

Nephroblastomas were observed in 50 Japanese eel reared at a farm for 5 to 9 months from 1989 after collection in the wild. The tumors, arising from the trunk kidney near the anus, were noted externally as abdominal swellings and varied in size from 30 to 75 mm in maximum diameter. Most were elastic, solid, and well encapsulated. Histologically, the nephroblastomas were composed of combinations of three main tissue elements. Spindle- or oval-shaped cells resembling human blastema cells were observed in most tumors to some larger or smaller degree. Although variation was evident from tumor to tumor, and even within the same tumor, the most common histological type was epithelial with formation of alveolar nests, the cells sometimes being arranged in tubular structures simulating normal renal tubules. A muscle tissue element with distinct cross-striations was also observed. Liver metasteses were found in one case. Histological examination of apparently normal kidneys from 100 eels revealed one early-stage nephroblastoma.

The cause of these tumors is unclear, although they have been discovered with increasing incidence after the spread of indoor eel culture with raised water temperatures (26-27°C) in Japan. Environmental factor(s) associated with the new aquaculture method may thus play a role in their occurrence.

**INTRODUCTION**

Kidney tumors occur commonly among the teleosts, especially in salmonid fishes, being next in frequency to liver tumors (1, 2). However, neoplasms of the kidney seem to be rather rare in the Anguilloid fishes (1-3), with only four previous reports documenting their occurrence (3-6). One adenocarcinoma was described by Schmey (4) in 1911, one adenoma by Plenh (5) in 1924, and one nephroblastoma by Ghittino et al. (6) in 1985 in the European eel, *Anguilla anguilla*. A nephroblastoma in one Japanese eel, *Anguilla japonica*, was recorded in 1981 in the Registry of Tumors in Lower Animals (3). More recently, 13 nephroblastomas in Japanese eel were reported by Miyazaki and Hyakkoku (7). The purpose of the present paper is to discuss the features of nephroblastomas in eel and those in humans on the basis of biological, histological, and ultrastructural observations of 50 cases of nephroblastomas in Japanese eel reared at a farm. Included was the result of a search for early-stage nephroblastomas in apparently normal Japanese eel kidneys.

**MATERIALS AND METHODS**

Fifty tumor-bearing Japanese eel (range of lengths, 400-740 mm) and 100 apparently normal Japanese eel (range of lengths, 450-630 mm) were obtained from a farm in Shizuoka Prefecture, Japan, during the period from August 1988 to September 1989 (Table 1). Kidney tumors were evident externally in fish which had been reared for 5 to 9 months at the farm from elvers caught on the Pacific coast of Shizuoka Prefecture in either March or December 1988. Similarly, 100 apparently normal eels (Table 2) reared at the same farm were randomly collected and transferred to the Cancer Institute. Tubifex was given for 1 or 2 weeks at the elver stage. Food was then switched gradually to cod meal (white meal) or sardine meal (brown meal) according to the growth. Daily food intake was determined as 1 or 2% of body weight (1 ton of eel consisted of approximately 4000 fish near the time of shipment). The eels were kept in circulating and well-oxygenated under-ground well water ponds covered with vinyl at 26-27°C throughout the year, and new water was at times supplied for partial water change, to maintain water quality.

All tumor-bearing eels and normal eels used in this study were immersed in ice-chilled water for immobilization and sacrificed by decapitation. The tumors and normal kidney tissues with other internal organs were fixed in 10% neutral formaldehyde solution and embedded in paraffin. Paraffin blocks were sectioned at 3-4 μm, and sections were stained with hematoxylin and eosin with periodic acid-Schiff and by phosphtungstic acid hematoxylin staining for muscle.

Six kidney tumors were examined by electron microscopy. Immediately after dissection, pieces of tissue were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) for 2 h at 0°C, postfixed in 1% osmium tetroxide in phosphate buffer, dehydrated by passage through a graded series of concentations of ethanol, and embedded in Epon. Ultrathin sections were contrasted with uranyl acetate and lead hydroxide and examined with a Hitachi HS-9 electron microscope.

Data on these Japanese eel and their tumors are summarized in Tables 1 and 2. Sexes of the fish were determined by histology of their sex organs. The Japanese eel used in this study demonstrated a male-biased ratio, as earlier described by Matsui (8).

**RESULTS**

**Gross Observations.** At autopsy, the kidney tumors were clearly observed as abdominal swellings near the anus (Fig. 1). They arose from the fused posterior region of the kidney (trunk kidney) and were never observed in the paired anterior kidney region (Fig. 2). The tumors varied in size from 30 x 15 x 10 mm to 75 x 25 x 15 mm and were all well capsulated (Fig. 3). Most were elastic and solid, although some were soft. Their cut surfaces were grayish-white. Some tumors had large cysts. Normal dark brown kidney tissues could sometimes be identified at the periphery of the tumor (Fig. 4).

**Histological Findings.** Histologically, tumor cells constituted most of the tissue masses, with normal kidney tissues occasionally being seen embedded as minor components. Most commonly tumors were composed of epithelial and muscular elements in different amounts, varying from focus to focus or from case to case. In some parts of tumors, irregular tubular structures with cuboidal or columnar epithelial cells resembling normal renal tubules were seen, and some of these were basophilic and others were eosinophilic. Epithelial cell nests were surrounded by proliferating blastema cells (Fig. 5). Primitive glomeruli were sometimes found within spaces lined by flattened cells somewhat similar to the parietal epithelium of Bowman's capsule (Fig. 6). In 38 of 50 tumor cases (76%), dark-stained spindle-shaped or oval cells resembling human blastema cells were observed around the tubules (Fig. 7). Strictly speaking, blastema cells were observed in most tumors to some larger or smaller degree. However, to clarify the histological...
Table 1  Histological type of Japanese eel nephroblastomas

<table>
<thead>
<tr>
<th>Predominating histological component</th>
<th>Number of tumors</th>
<th>Renal tubules (%)</th>
<th>No. of tumors with blastema cells (%)</th>
<th>Skeletal muscles (%)</th>
<th>Number of metastases (organ)</th>
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<tr>
<td></td>
<td>50</td>
<td>38 (76)</td>
<td>38 (76)</td>
<td>20 (40)</td>
<td>1 (liver)</td>
</tr>
</tbody>
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DISCUSSION

In 50 cases of nephroblastomas observed in wild-born Japanese eel reared on a farm, all developed in the posterior part of the kidney (trunk kidney), which is functional, with abundant neprons. The paired long, slender kidneys extend anteriorly from the trunk kidney. In the eel the anterior portion of the kidney is poorly developed (9). Although reptiles, birds, and mammals are characterized by the development of a posterior kidney (metanephros) which is functional throughout adult life (10), the mesonephros has a chiefly renal function in adult fish (9). In Anguillid fishes, the pronephros is a vestigial organ in elvers (11). The mesonephros has the chief renal functions in the elvers (11) and adults (9, 11). Therefore, the results would suggest that eel nephroblastomas originate from the mesonephros. However, in humans nephroblastomas were regarded to arise from the metanephric blastema (12). Histologically, the finding that eel tumor tissues included blastema-like cells, renal tubular cells, and skeletal muscle cells or stromal cells suggests essential similarities with nephroblastomas in humans (12). A histopathological study on 13 cases of eel tumor was made by Miyazaki and Hyakkoku (7). In our cases of eel nephroblastomas, Z bands were found in the muscle fibers by electron microscopy, as in human nephroblastomas (12). There have been no reports concerning the presence of striated muscle in nephroblastomas from fishes other than the Japanese eel (6, 13, 14). But the presence of smooth muscle was not clear in the eel nephroblastomas, as mentioned by Miyazaki and Hyakkoku (7). The existence of cartilage has been reported for nephroblastomas in one Japanese eel (7), one European eel (6), one striped bass, Morone saxatilis (13), and one rainbow smelt, Osmerus mordaks (14), as well as in humans (12). Electron microscopic observations revealed that the cilia have the 9 + 2 pattern in the tumorous tubular cells of eel nephroblastomas. In the normal adult fish kidney, cilia are always seen in the proximal tubules (15). Ito and Johnson (16) reported that cilia formation was observed in 8 of the 23 Wilms’ tumors and occasionally in normally developing proximal tubules. The cilia formation in the Wilms’ tumors was regarded as an expression of embryonal character of the tumors. Human nephroblastomas often metastasize, and the histological appearance of metastatic lesions is sometimes different from that of the primary tumor (12). In one eel nephroblastoma case where hepatic metastases were found, the metastases markedly differed in histological type from the main primary tumor component. Thus the histological and ultrastructural findings showed a striking similarity between nephroblastoma lesions in both humans and the Japanese eel. However, it must be borne in mind that embryologically the mesonephros is the functional kidney in fish (17).

In humans, nephroblastomas develop in children or infants (12). The present nephroblastomas, in contrast, were observed in fish which had been maintained in a pond for 5 to 9 months after collection from the wild as elvers. These elvers are believed to arrive at the Japanese coast by swimming from the Luzon area in the Philippines within 6 months after hatching (18). Therefore, the Japanese eel nephroblastomas can be regarded as developing within approximately 1 year. Thus while onset age seems to correspond to the period of infancy, eel adults may also be affected, and nephroblastomas in fish are not always embryonal tumors, as in humans (17).

In Japanese eel nephroblastomas, genetic influences may have been a contributory factor, as in human nephroblastomas, since these fish are considered to be relatively homogeneous in terms of genetic background (19). It must be pointed out, however, that the incidence of nephroblastomas has clearly increased with the spread of indoor eel farming using circulating water.
Fig. 1. Eel with nephroblastomas demonstrating abdominal swellings in the anal region. × 0.5.

Fig. 2. Normal eel kidney showing the posterior portion of the kidney (trunk kidney, arrowhead) and the paired anterior kidneys (arrows). × 1.0.

Fig. 3. Nephroblastoma arising from the trunk kidney, × 1.3.

Fig. 4. Cut surface of an eel nephroblastoma. Normal dark brown kidney can be identified at the periphery, × 1.5.

Fig. 5. Microscopic overview of nephroblastoma showing epithelial cell nests and surrounding blastema cells. H&E, × 90.

Fig. 6. Section of an eel nephroblastoma demonstrating a blastema aggregate and nests of tubular differentiation. The glomerular profiles are not necessarily within tubules but within epithelium-lined spaces possibly equivalent to Bowman’s capsules. H&E, × 90.

Water adjusted to 25–26°C and dense population culture conditions. Therefore, environmental factors may also be responsible. First of all, infection with viruses can be considered as one possible etiological factor. Nephroblastomas can be induced in chicken by the chicken retrovirus, NK 24, isolated from a chicken nephroblastoma (20) and a Birnavirus group-type virus was isolated from the internal organs of Japanese eel with nephroblastomas by Ueno et al. (21) in 1984. However, they failed to induce nephroblastomas in eel following injection of the virus (21).

The possibility of genesis of carcinogens or promoters in the waters cannot be excluded. An maximum of 6 ppm nitrous acid was actually measured in eel farm culture water (22), a relatively high level compared to well water (maximum 0.08 ppm) from the same region (22). According to Yamagata and Niwa (23), nitrous oxide, at concentrations of 1, 3, or 10 ppm, caused neither acute nor chronic toxicity in Japanese eels, but 30 ppm caused growth inhibition and hypochromemia. While the carcinogenicity of sodium nitrite itself has not been clarified, this chemical can act as a precursor for carcinogenic N-nitroso compounds in combination with secondary amines in food or in the body (24). This may be of relevance, since Ashley (25) succeeded in inducing nephroblastomas in the adult rainbow trout, Oncorhynchus mykiss, using dimethylnitrosamine. Grieco et al. (26) failed to produce nephroblastomas with dimethylnitrosamine but confirmed that dimethylnitrosamine was a potent liver carcinogen in rainbow trout.

In the European eel, the formation of new nephrons occurs following injection of mammalian prolactin (27). A single injection of 500 mg/kg hexachlorobutadiene also induces new nephron development in adult goldfish kidneys (28). This potential for differentiation during the adult stage would suggest that low levels of carcinogens in the aquatic environment may initiate nephroblastomas even in adult fish. The present results are in line with the report of Ghittino et al. (6) of a nephroblastoma occurring in a silver European eel, judged to be an adult from its color and length.

In ponds covered with vinyl, the warm water adjusted to 26–27°C may enhance carcinogenic or nephrotoxic effects. For example, Kyono and Egami (29) noted that liver tumorigenesis in the medaka, Oryzias latipes, was accelerated when the temperature was high. However, the reason why nephroblastomas in the Japanese eel have increased with the spread of indoor eel culture remains obscure. A number of laboratories have recently reported that activation of oncogenes may be essential for the induction of tumors in mammals (30), and fish have proved to be no exception (31, 32). Phylogenetic studies have revealed that oncogenes are widely distributed in various organisms, including fish (32–37). Interest is now being concentrated on whether eel nephroblastoma development may also be regulated by antioncogenes as isolated from human nephroblastomas by Rose et al. (38). The eel nephroblastomas could be a potentially...
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Fig. 7. Section of a nephroblastoma illustrating blastema cells surrounding tubular structure. H&E, x 200.

Fig. 8. Section of a nephroblastoma in an eel showing muscle tissue elements with distinct striations. Stained by phosphotungstic acid hematoxylin. x 900.

Fig. 9. Section of the liver of an eel containing a metastatic focus with features of renal tubuli admixed with blastema cells. H&E, x 200.

Fig. 10. Section of an apparently normal kidney containing a microscopic nephroblastoma (arrows). H&E, x 100.

Fig. 11. Electron micrograph of an eel nephroblastoma demonstrating the blastema cells, x 7,500.

Fig. 12. Electron micrograph of an eel nephroblastoma illustrating tumors tubular epithelial cells with cilia protruding into the lumen and abortive striated muscle cells. x 7,500.

important model for helping to elucidate the molecular pathogenesis of Wilms's tumors.

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