**Spontaneous Olfactory Neuroepithelioma in a Domestic Medaka (Oryzias latipes)**

Chikao Torikata, Makio Mukai, and Keizo Kageyama

Department of Pathology, Keio University School of Medicine, Tokyo 160, Japan

---

**ABSTRACT**

Tumors of the central nervous system in fish are rare, and only six cases of spontaneous olfactory neuroepithelioma have been reported. This is the seventh case, found in a medaka, *Oryzias latipes*. The tumor was noted near the right olfactory orifice and finally measured 1.5 mm in diameter. Histologically the tumor consisted of undifferentiated neuroblasts forming a few true rosettes. Mitosis was frequently observed. Tumor cells stained diffusely for neuron-specific enolase and sporadically for neurofilament proteins by immunohistochemical procedures. Additionally a few large tumor cells were positively stained for S-100 protein. Electron microscopy revealed that the tumor cells had extended cytoplasm in which parallel neurotubules and a few neuroendocrine granules were noted. In the perinuclear region, bundles of intermediate filaments and neuroendocrine granules were seen. Single cilia and a pair of centrioles were occasionally found, but no ciliated cells were found in this tumor. Some large tumor cells contained electron-dense intracytoplasmic inclusions which showed a crystalloid structure by high-magnification electron microscopy; however, this type of crystalloid has never been reported in neuronal tumors.

**INTRODUCTION**

Although various types of neoplasms in fish have been reported and reviewed (1, 2), tumors of the central nervous system have rarely been reported, and there are only 6 previous reports of spontaneous olfactory neuroepithelioma in fish: one in a gilthead, *Sparus aurata* (3); one in a bloater, *Coregonus hoyi* (4); and one in a domestic carp, *Cyprinus carpio* (5), all of which became somewhat emaciated and refused to take food. The fish was anesthetized by injection of pentobarbital sodium solution, and a small piece of the tumor mass was cut for electron microscopic observation. The body of the fish was fixed in 10% formalin solution, and after a mild decalcification procedure, it was cut sagittally and embedded in paraffin. Three-μm sections were stained with hematoxylin-eosin, periodic acid-Schiff reagent, Mallory stain, silver impregnation, and Grimelius, Fontana-Masson, and Bodian stains. The avidin-biotin complex immunoperoxidase technique (12) (Vectastain; Vector Laboratories, Burlingame, CA) was used to stain sections for NSE2 (IBL, Maebashi, Japan; dilution, 1:300), S-100 protein (DAKOPATTS, Glos-trup, Denmark; dilution, 1:200), and neurofilament proteins (Amer-sham, Buckinghamshire, England; dilution, 1:200). These antisera can be applicable not only to human tissue but also to fish tissue, judging from out-control experiments using normal human and fish tissues.

For electron-microscopic observation, the tissue was directly fixed with 1% glutaraldehyde and postfixed with 1% osmium tetroxide. After dehydration with graded alcohols and acetone, the tissue was embedded in Spurr's resin. Ultrathin sections were cut, doubly stained with uranyl acetate and lead citrate, and examined with a JEOL Model 100C electron microscope at 100 kV.

---

**RESULTS**

At autopsy, a smooth-surfaced elevation measuring 1.5 mm in diameter was noted on the right side of the snout between the eyeball and the upper lip (Fig. 1). The cut surface of the tumor was edematous and not pigmented. After formalin fixation, the tumor became firm and grayish white. The tumor occupied the olfactory chambers, but no distant metastases were noted.

**Light Microscopic Observation.** The tumor occupied mainly the right olfactory chambers, but showed invasive growth into the surrounding tissues with destruction of the bone (Fig. 2). The tumor was highly cellular and was composed chiefly of two cell types, small undifferentiated cells and large polygonal cells containing a considerable number of mitoses; and they were separated by delicate fibrovascular tissue. Numerous cytoplasmic processes extended from large polygonal tumor cells and formed fibrillar areas in the periphery of the nests. A few organoid structures, including a few true rosettes and acinar structures, were noted, but no ciliated cells were seen in the luminal surface (Fig. 3). Both types of the neoplastic cells were diffusely stained for NSE, and some scattered large neoplastic cells were stained for S-100 protein (Fig. 4). Grimelius stain revealed scattered argyrophil granules mainly in the perinuclear region of the large polygonal cells (Fig. 6A), but they were not stained by Fontana-Masson stain. By immunostaining for neurofilament proteins, positive reaction products were detected within the cytoplasm (Fig. 7A).

**Electron-microscopic Observation.** The tumor cells had clear cytoplasm with abundant ribosomes, mitochondria, an endoplasmic reticulum, and a prominent Golgi apparatus. The nucleus was irregularly indented and contained a small but prominent nucleolus. Mitotic cells were not infrequently observed. A sheet of neoplastic cells was separated by bundles of the fibroblastic cells, sometimes containing a capillary (Fig. 5). These neoplastic cells had extended cytoplasm and formed bundles of fine cytoplasmic processes, like neurites, in which...
there were neurotubules parallel to the long axis and a few membrane-bound cored vesicles 170 nm in diameter. Some neoplastic cells contained numerous neuroendocrine granules in the perinuclear region (Fig. 6B). In addition to neurotubules, the neoplastic cells contained bundles of intermediate-sized filaments in the cytoplasm and occasionally were filled with aggregates of the filaments. These filaments were about 10 nm in diameter (Fig. 7B). Some neoplastic cells contained a pair of centrioles in close relationship to the Golgi apparatus, and a single cilium sprouted from one of them. The centrioles consisted of 9 peripheral triplets in a ring, and a regular alar-like structure was noted inside the right (Fig. 8). True rosettes consisted of several large polygonal cells, and between neighboring cells junctional complexes were noted near the luminal surface (Fig. 9). A single cilium was also noted on the luminal surface of the rosette, but ciliated cells were never found in this tumor. Some large neoplastic cells contained electron-dense intracytoplasmic inclusions in the perinuclear region. By high-magnification electron microscopy, membrane-bound crystallloids and ribbon-like aggregates probably derived from the unit membrane were detected in the cytoplasm (Fig. 9, inset). These

---

**Fig. 1.** Tumor of a female hi-medaka. The tumor mass is protruding near the right olfactory orifice between the eyeball and the upper lip. The tumor is about 1.5 mm in diameter.

**Fig. 2.** A low-magnification light micrograph of a sagittal section through the tumor. The tumor occupies the olfactory chambers and is extremely hypercellular. H&E, × 15.

**Fig. 3.** Histology of the olfactory tumor. The tumor is highly cellular and consists of sheets of small undifferentiated cells without architectural arrangement and fairly large polygonal cells with organoid structures including true rosettes. The tumor shows invasive growth with destruction of the bone. H&E, ×100 (A) and ×400 (B).

**Fig. 4.** Immunostaining for NSE and S-100 protein. The neoplastic cells are diffusely stained for NSE (A). Large polygonal neoplastic cells are occasionally stained positively for S-100 protein (B). Avidin-biotin complex method, × 400.
crystalloid structures in neurogenic tumors have never before been reported; therefore the fine structure of these crystalloids will be presented elsewhere.

DISCUSSION

Since the original report on olfactory neuroepithelioma (esthesioneuroepithelioma) by Berger et al. (13) was published in 1924, this type of tumor has rarely been found in humans. The histological features of these tumors vary from case to case; however, closely packed undifferentiated neuroblasts including areas showing tangled neurofibrils or more regular sheets are characteristic and, in some cases, true rosettes are present (14). On the basis of the differentiation of the tumor, two histological categories, olfactory-neurocytoma and olfactory-neuroepithelioma, have been proposed (15). Electron microscopy revealed...
that this tumor is characterized by the presence of numerous cytoplasmic processes, neuroendocrine granules, neurotubules, and neurofilaments, like a neuronal tumor (16, 17). The formation of true rosettes in olfactory neuroepithelioma is thought to be characteristic, but in fact, true rosettes have been found infrequently, and ultrastructural study revealed that no ciliated cells are involved in human cases. In spite of intensive ultrastructural and histochemical studies, the histogenesis of this type of tumor has not been resolved completely. Recently a new clinicopathological entity in humans, sinonasal undifferentiated carcinoma, distinct from olfactory neuroepithelioma has been proposed (18, 19). In experimental animal models, olfactory epithelial tumors have been induced by chemical carcinogens in the hamster (20) and in the rat (21), indicating their origin...
in the nerve cell in the olfactory epithelium. However, Vollrath et al. (21) reported that the undifferentiated tumor cells lacked intermediate filaments, and only a few tumor cells were positively stained for neurofilament protein.

Other than human cases, only one case of spontaneous olfactory neuroepithelioma in mammals has been reported; it was in a cynomolagus monkey (Macaca fascicularis) (22). In amphibians, a transplantable tumor of this type in an axolotl, Ambystoma (formerly Siredon) mexicanum, was described by Brunst (23). In fish, 6 cases have been previously reported; this is the seventh case. The first report of the tumor in fish, in a gilthead by Thomas in 1932 (3), could not be reviewed; however, in the second case, in a bleaker (4), many well-developed rosettes with olfactory lashes and crypts were detected by light microscopy. In the third case, in a nishiki-goi, a domestic carp, by Ishikawa et al. (5) nonmyelinated axons and numerous cilia projecting into the lumen of the rosette were present, however, in some cases no neoplastic cells contained a pair of centrioles, and a single cilium projected from one of them; no ciliated cells were found. The ultrastructure of the neoplastic cells in the medaka was similar to that of human cases, showing numerous cytoplasmic processes, neuroendocrine granules, neurofilaments, and neurotubules. Additionally S-100 protein-positive cells were seen among the large polygonal neoplastic cells, indicating that some neoplastic cells differentiate into Schwann cells as found in human cases (24, 25).

In neuroblastomas in the human, unusual ultrastructural findings have rarely been reported (26); however, there are no crystalloids in the neuronal tumors. The crystalloids found in this case are similar in size and substructure to the crystalloids found in alveolar soft-part sarcoma (27, 28), and these findings indicate the possibility of a close relationship to some cytoskeletal proteins.

ACKNOWLEDGMENTS

We are grateful to Dr. John C. Harshbarger, National Museum of Natural History, Smithsonian Institution, Washington, DC, for his encouragement and review of this manuscript. We also thank K. Takeichi for his excellent use of photographic technique.

REFERENCES


Spontaneous Olfactory Neuroepithelioma in a Domestic Medaka (Oryzias latipes)

Chikao Torikata, Makio Mukai and Keizo Kageyama


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/49/11/2994

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.