Pathological Features of Olfactory Neuroblastoma in an Axolotl (Ambystoma mexicanum)

Chieko SHIODA1)*, Kazuyuki UCHIDA1) and Hiroyuki NAKAYAMA1)

1)Department of Veterinary Pathology, Graduate School of Agricultural and Life Science, The University of Tokyo, 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113–8657, Japan

(Received 3 March 2011/Accepted 12 April 2011/Published online in J-STAGE 26 April 2011)

ABSTRACT. A one-year-old, female Mexican axolotl (Ambystoma mexicanum) had a rough-surfaced, polypoid, pink tumor mass of approximately 10 mm in diameter in the oral cavity. Histologically, the tumor extended from the ethmoturbinate region and into the oral cavity and had replaced some of the maxillary bone tissue. The tumor mass was composed of a lobular architecture of small round-shaped tumor cells with occasional Flexner-Wintersteiner-like rosette formation. There were no metastatic lesions in the other organs. Immunohistochemically, the tumor cells were partly positive for several neural markers (class III beta-tubulin, S-100 protein, and doublecortin) and intensely positive for an epithelial marker (cytokeratin AE1/AE3). These results suggest that the present tumor originated from neuroectodermal tissue. Considering the location and histological and immunohistochemical features of the tumor, a diagnosis of olfactory neuroblastoma was made.

KEY WORDS: amphibian, axolotl, olfactory neuroblastoma.

The axolotl is a urodele amphibian, and like other amphibians, is used as an experimental animal for cancer research, regenerative biology, and immunology [9]. Recently, the axolotl has become a popular pet in Japan. Although there are similarities in the cellular structures of humans and amphibians, spontaneous tumors are rare in amphibians [1, 5]. Regarding the spontaneous tumors that occur in axolotls, melanoma [10], epithelioma [1], neuroepithelioma [2], lymphangiosarcoma [8], mast cell tumor [6], and telatoma [3] have been reported. However, there are few detailed studies about these tumors. In this report, the pathological features of a spontaneous tumor located between the oral and nasal regions in an axolotl are described.

A one-year-old, female Mexican axolotl (Ambystoma mexicanum) had a papillary tumor mass in the oral cavity. The mass was rough-surfaced, polypoid, pink in color, and approximately 10 mm in diameter (Fig. 1). In a cytological examination with fine needle aspiration (FNA), round to pleomorphic cells with a clear cytoplasm and an atypical round nucleus were observed. Two months later, the axolotl died. In necropsy, the tumor was found to be attached to the maxilla and had not invaded the brain. No significant lesions were observed in other organs. Samples were collected from the tumor mass as well as the maxilla and other visceral organs including the brain, spinal cord, heart, lung, stomach, intestines, liver, pancreas, kidneys, and ovaries. Tissue samples were fixed in 10% formalin solution and embedded in paraffin using a routine procedure. Some selected sections were also stained with Masson’s trichrome and phosphotungstic acid hematoxylin (PTAH).

Immunohistochemistry was performed by the Envision polymer method (Dako-Japan, Kyoto, Japan) using standard reagents. The primary antibodies used were mouse monoclonal antibodies against class III beta-tubulin (1:1000, Promega Corporation, Madison, WI, U.S.A.) and cytokeratin AE1/AE3 (1:50, Dako-Japan), rabbit antisera against S-100 protein (1:400, Dako-Japan), and goat antiserum against doublecortin (DCX, 1:400, Santa Cruz Biotechnologie, Santa Cruz, CA, U.S.A.). The reaction products were visualized by reacting them with 3,3’-diaminobenzidine (DAB).

Histologically, the tumor mass extended from the ethmoturbinate region and into the oral cavity and had replaced some of the maxillary bone tissue (Fig. 2). The mass was unencapsulated and composed of a lobular architecture (Fig. 3). The lobes consisted of nests or sheets of small tumor cells with little cytoplasm, distinct cytoplasmic processes, and small round nuclei. These cells were arranged in cords and often formed tubular structures or multi-layered rosettes (Flexner-Wintersteiner-like rosettes). On the apical surface of the lumen, eosinophilic fibrillary structures were detected (Figs. 3 and 4). Mitotic figures were occasionally found. No tumor lesions were found in the brain.

The results of the immunohistochemical analysis are summarized in Table 1. The normal nervous tissues of the axolotl were positive for neuronal markers (class III beta-tubulin and S-100 protein), a glial marker (GFAP), and an epithelial marker (cytokeratin AE1/AE3, Table 1). The tumor cells were partly positive for class III beta-tubulin (Fig. 5a), S-100 protein, and DCX (Fig. 5b), but negative for GFAP (Fig. 5d). Almost all tumor cells were intensely positive for cytokeratin AE1/AE3 (Fig. 5c). The nervous tis-
The present findings indicate that the tumor originated from neuroectodermal tissue. A previous report described a neuroepithelioma in the roof of the mouth of an axolotl [2]. While the cytological composition and growth patterns of the present and previously reported tumors are similar, the present tumor may have arisen in the nasal cavity and have originated from olfactory neurons. Thus, we made a diag-
sues of the axolotl were negative for neurofilament, nestin, NSE, synaptophysin, vimentin, MAP2, and chromogranin A.

Fig. 1. A rough-surfaced, polypoid, pink-colored tumor mass is found within the oral cavity (arrow). The tumor mass partly protrudes outside the nasal cavity (arrowhead).

Fig. 2. The tumor extends from the ethmoturbinate region and into the oral cavity and has replaced some of the bone tissue of the maxilla. N: Nasal cavity, O: Oral cavity, T: Teeth. HE. Bar=2 mm.

Fig. 3. The tumor is composed of a lobular architecture. HE. Bar=200 μm.

Fig. 4. The tumor cells form tubular structures or multi-layered rosettes (Flexner-Wintersteiner-like rosettes). There are eosinophilic fibrillary structures on the apical surface of the lumen. HE. Bar=30 μm.

Fig. 5. The tumor cells are partly positive for class III beta-tubulin (a) and DCX (b) and intensely positive for cytokeratin AE1/AE3 (c), but they are negative for GFAP (d). Immunostaining. Bar=30 μm.
Olfactory neuroblastomas are rare tumors arising from the olfactory mucosa, but have been reported in cats, dogs, and horses [4, 7] as well as humans. The olfactory neuroblastomas found in dogs and cats were composed of circumscribed lobules or nests of tumor cells with little cytoplasm, round nuclei, and delicate fibrillary cytoplasmic processes. The tumor cells often form rosettes (Homer-Wright and/or Flexner-Wintersteiner-like rosettes) or gland-like structures [11]. The tumor grows slowly and frequently destroys the nasal turbinates. Immunohistochemically, the canine and feline olfactory neuroblastoma cells were positive for several neuroendocrine markers, including synaptophysin and partly positive for epithelial markers including cytokeratin [7]. The tumor cells of the present axolotl case were positive for neuronal markers, class III beta-tubulin, S-100 protein, and DCX, and an epithelial marker, cytokeratin AE1/AE3.

Considering the location of the tumor and its histological and immunohistochemical features, the present tumor was diagnosed as olfactory neuroblastoma. Since the morphological features of the present case are similar to those of a previous case that was diagnosed as oral neuroepithelioma [2], the two tumors might have common neuroectodermal tissue origins between the nasal and oral cavities.

REFERENCES


Table 1. Immunoreactivity of the tumor cells and normal nervous tissue of the axolotl

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Neoplastic cells</th>
<th>Normal neurons</th>
<th>Normal glial cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class III beta-tubulin</td>
<td>+</td>
<td>++</td>
<td>–</td>
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<tr>
<td>S-100 protein</td>
<td>+</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>Doublecortin (DCX)</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>GFAP**</td>
<td>–</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>Cytokeratin AE1/AE3</td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
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* Intensity of immunoreactivity: –; negative, +; <50% cells are positive, and ++; >50% cells are positive. **Glial fibrillary acidic protein.