Neuromastoma of the hard palate mucosa in an Australian green tree frog (Litoria caerulea)

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ABSTRACT. A hard palate mass was surgically removed from an Australian green tree frog (Litoria caerulea) and examined pathologically. The tumor consisted of sheets of small cells arranged in a tubular structure and cords or rosettes with fibrovascular stroma. Immunohistochemically, neoplastic cells were diffusely positive for cytokeratin and neuron-specific enolase and partially positive for S-100 and doublecortin. These findings indicate that the tumor originated from the neuroectodermal tissue. Based on these findings, the tumor was classified as a neuromastoma (neuroepithelioma). Sensory cells located in the hard palate of the frog were considered to be the origin of the tumor. The frog died after going through 3 surgeries and experiencing difficulties closing its mouth.

KEY WORDS: frog, neuroepithelioma, oral palate
occasionally arranged in glandular, trabecular, and rosette patterns. Mucinous material was often observed in the lumen of the glandular structures. The rosettes were predominantly of the Flexner-Wintersteiner type, exhibiting central canals, while some were the Homer-Wright type with fibrillary structures in the center (arrowheads) are pictured. Scale bar=50 μm. (b) Neoplastic cells are polygonal in shape with scant eosinophilic cytoplasm and round to oval-shaped, hyperchromatic nuclei. Scale bar=30 μm; Hematoxylin and eosin (HE).

Fig. 2. Histological findings of the tumor. (a) The tumor is highly cellular with a lobular pattern surrounded by thin connective tissue. Flexner-Wintersteiner rosettes forming ductal structures (arrow) and Homer-Wright rosettes with fibrillary structures in the center (arrowheads) are pictured. Scale bar=50 μm. (b) Neoplastic cells are polygonal in shape with scant eosinophilic cytoplasm and round to oval-shaped, hyperchromatic nuclei. Scale bar=30 μm; Hematoxylin and eosin (HE).
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The formation, one of the histological characteristics of neuroectodermal tumors, was observed. The tumor cells were positive for DCX, which is a marker for immature neurons/neuroblasts [7]. In addition, the tumor was positive for NSE, S-100, and CK.

Sensory cells in amphibians are called neuromast cells, and the neoplastic counterpart is referred to as a neuromastoma [6], though the nomenclature of “neuroepithelioma” has been also used in some reports [6]. Neuromastomas (neuroepitheliomas) share similar histological features as olfactory neuroblastomas in amphibian [6, 12]. Histologically, tumor cells form variably sized nests and lobes with Flexner-Wintersteiner type rosettes and fine fibrovascular stroma. Considering the tumor location and histological features, the lesion of the present case was further diagnosed as neuromastoma (neuroepithelioma) of the hard palate.

It is known that this species can live for over 16 years in captivity [8]. In the present case, the frog died after going through 3 surgeries and experiencing difficulties closing its mouth. Anorexia and respiratory distress due to the tumor mass are the likely causes of death for the frog. The biological behavior of NTs in amphibians remains unclear. In a previous study, a NT with a prolonged clinical course in an axolotl suggested a benign nature [13]. By contrast, the present study and a report by Shioda et al. suggest that NTs can be malignant in nature [12]. Further research is necessary to determine the biological behavior of this tumor in amphibians.

To date, there are few studies describing NTs in amphibians [5, 6, 12]. This is the first report describing the clinical, histological, and immunohistochemical features of a neuromastoma (neuroepithelioma) in a frog, which likely originated from the neurosensory cells in the hard palate.

Table 1. Immunohistochemical findings of normal and tumor tissues of the frogs

| Antibodies** | Immunoreactivity* | Neoplastic cells a) | Normal mucoepithelial cells of present case a) | Normal neuronal cells of the Japanese brown frog b) |
|--------------|------------------|---------------------|---------------------------------|---------------------------------|
| Cytokeratin  | ++               | ++                  | −                               |                                  |
| NSE          | ++               | +                   | +                               | +                               |
| DCX          | +                | +                   | +                               | +                               |
| S-100        | +                | +                   | +                               | +                               |

Intensity of immunoreactivity*: −, negative; +, <50% positive cells; ++, ≥50% positive cells. **NSE, neuron specific enolase; DCX, doublecortin. a) Present case, Australian green tree frog (Litoria caerulea). b) Positive control, Japanese brown frog (Rana japonica).

Fig. 3. Immunohistochemical findings of normal mucosal epithelium and neoplastic cells. (a) Normal mucosal epithelium is strongly positive for cytokeratin (CK). Scale bar=20 µm. (b) Neuron-specific enolase (NSE)-positive cells are observed in the normal mucosal epithelium (arrowhead). Scale bar=20 µm. (c) Doublecortin (DCX)-positive cells are observed in the normal mucosal epithelium. Scale bar=20 µm. (d) Tumor cells are diffusely positive for CK. Scale bar=30 µm. (e) Tumor cells are diffusely positive for NSE. Scale bar=30 µm. (f) Tumor cells are occasionally positive for DCX and show apical cytoplasmic processes. Scale bar=30 µm; 3,3′-Diaminobenzidine and hematoxylin counterstain.
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