



NOTE

Pathology

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

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The Australian green tree frog (*Litoria caerulea*), known as the White tree frog, is a tree frog native to Australia and New Guinea. It is one of the most popular pet frogs throughout the world. Compared to that of mammals, birds, and fish, neoplasia of amphibians is seldom reported [1]. Most of the reported cases of amphibian tumors are epithelial tumors of the skin, such as squamous cell carcinoma, adenoma, adenocarcinoma, papilloma, and epithelioma [2–4, 6]. There are a few studies regarding neuroblastic tumors (NTs), including 2 cases of neuroblastoma in axolotls [5, 12]. In mammals, only a few cases of oral NTs have been reported in humans [10, 15] and canines [9]. Here, we describe the pathological findings of a neuromastoma arising from the hard palate of a captive adult Australian green tree frog.

An adult male Australian green tree frog of unknown age, approximately 10 cm in body length and 93 g in weight, was referred to a veterinary clinic for a firm mass on the right side of the hard palate that had been gradually enlarging for several weeks (Fig. 1a). The frog did not show any clinical signs associated with the mass. A computed tomography (CT) scan showed a focal, expansile mass in the right lateral hard palate (Fig. 1b). The nasal bone and orbit were intact. The mass was surgically resected. Grossly, the resected tissue was firm, pinkish, had a rough surface, and measured 1 cm in diameter. The neoplastic mass recurred and reoperations were performed 1 month and 2 months after the initial surgery. Three months after the initial surgery, the frog developed a problem closing its mouth due to the recurrent mass and sustained a weight loss of 15 g. The frog died 4 months after the initial surgery; however, necropsy was not performed.

All 3 excised tumor tissues were immediately fixed in 10% neutral buffered formalin and routinely embedded in paraffin wax. Sections of 4 μm thick were stained with hematoxylin and eosin, periodic acid-Schiff (PAS), and Grimelius silver nitrate. Serial sections were also subjected to immunohistochemical analysis using the Envision polymer (Dako, Tokyo, Japan) and 3, 3'-diaminobenzidine tetrahydrochloride as a chromogen. The following primary antibodies were used: mouse anti-cytokeratin (CK) (clone AE1/AE3, ready to use, Dako), rabbit anti-neuron-specific enolase (NSE) (ready to use, Nichirei, Tokyo, Japan), rabbit anti-S-100 (1:500, Dako), and goat anti-doublecortin (DCX) (1:200, Santa Cruz Biotechnology, Dallas, TX, U.S.A.). For antigen retrieval, the sections were treated at 121°C for 10 min in citrate buffer (pH 6.0). Brain tissue from a necropsy case involving a Japanese brown frog (*Rana japonica*) was used as a positive control. For a negative control, the primary antibody was omitted.

On microscopic examination, a non-encapsulated tumor was located in the submucosa of the hard palate (Fig. 2a). The tumor showed a lobular pattern separated by thin fibrous connective tissue (Fig. 2a). The lobules consisted of sheets of small tumor cells

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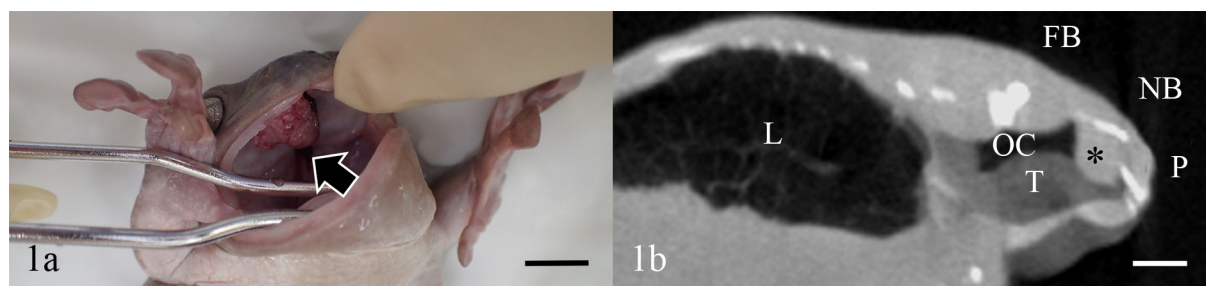


Fig. 1. Gross and computed tomography (CT) findings of the tumor. (a) A mass protruding from the oral mucosa on the palate of the upper jaw (arrow). Scale bar=1 cm. (b) A CT sagittal image shows a mass in the upper oral cavity (asterisk). The structure of the adjacent palatal bone is intact. Abbreviations: FB, frontoparietal bone; L, lung; NB, nasal bone; OC, oral cavity; P, premaxilla; T, tongue. Scale bar=1 cm.

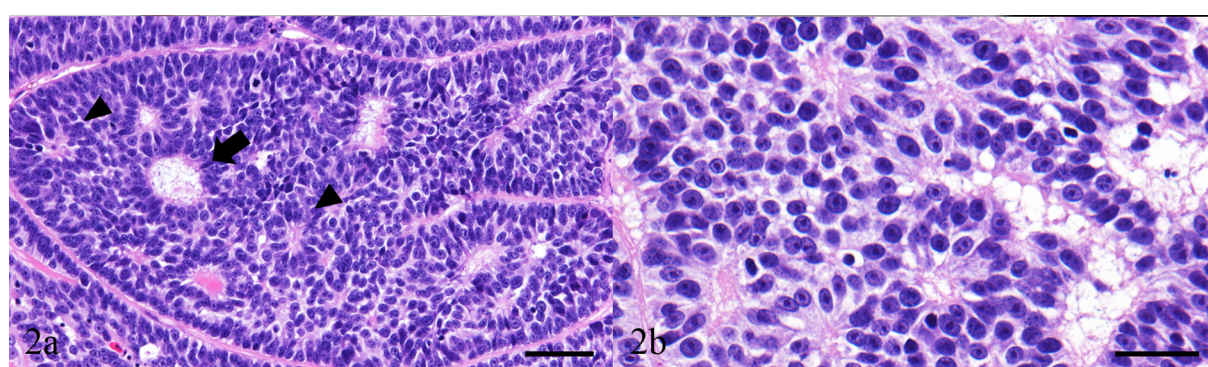


Fig. 2. Histological findings of the tumor. (a) The tumor is highly cellular with a lobular pattern surrounded by thin connective tissue. Flexner-Wintersteiner rosettes forming ductal structures (arrow) and Homer-Wright rosettes with fibrillary structures in the center (arrowheads) are pictured. Scale bar=50 μ m. (b) Neoplastic cells are polygonal in shape with scant eosinophilic cytoplasm and round to oval-shaped, hyperchromatic nuclei. Scale bar=30 μ m; Hematoxylin and eosin (HE).

occasionally arranged in glandular, trabecular, and rosette patterns. Mucinuous 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 (Fig. 2a). The tumor cells were pleomorphic with scant eosinophilic cytoplasm, round to oval-shaped hyperchromatic nuclei, and small nucleoli (Fig. 2b). Additionally, multinucleated cells were occasionally observed. The number of mitotic figures of the tumor cell was 8 per 10 high power fields. Apoptotic bodies were frequently observed. Multifocal to diffuse hemorrhage and necrosis were present. Vascular invasion of the tumor tissue was absent. The tumor cells were negative for PAS and Grimelius silver nitrate. In addition, the tumor cells were observed at the surgical margins of the primary tumor. All 3 resected tumor tissue samples showed similar histopathological findings.

The results of the immunohistochemical examinations are summarized in Table 1. The normal neuronal cells of the Japanese brown frog were positive for the neuronal cell markers (NSE, DCX, and S-100) and negative for an epithelial cell marker (CK). The normal mucosal epithelial cells in the palate of the Australian green tree frog in the present case were immunopositive for CK (Fig. 3a), while the NSE-, DCX-, and S-100-immunohistochemistries revealed randomly scattered immunopositive cells that were polygonal-shaped and had apical cytoplasmic processes (Fig. 3b and 3c). In the tumor tissue, the neoplastic cells were diffusely immunopositive for CK (Fig. 3d) and NSE (Fig. 3e), while DCX- (Fig. 3f) and S-100-positive cells were scattered. Branching processes extended from the cell body of the DCX-immunopositive cells (Fig. 3f). Based on the histological and immunohistochemical findings, the tumor was classified as a NT.

The diagnosis of NT indicates a ganglionic or neuroepithelial origin. In the present case, the tumor was confined to the oral cavity without any space-occupying lesions in the nasal cavity or destruction of the nasal or palatine bones; therefore, it is likely that the tumor originated from the hard palate rather than the nasal tract. The palate mucosa of the frog consisted of pseudostratified columnar respiratory-like epithelium, including ciliated cells, mucous and serous cells, intermediate cells, basal cells, and also patches of vomeronasal epithelium with sensory receptors and supporting cells [11]. It is known that autonomic ganglion cells are not present in the oral mucosa of healthy adult frogs [14]. Therefore, the neurosensory cells of the palate mucosa testing positive for neuronal markers (S-100, NSE, and DCX) were considered to be the origin of the present tumor. Taste buds were also considered as a possible origin; however, normal taste bud cells did not express any of the neuronal markers used in this study.

In the present case, neoplastic tissue was mainly composed of small round cells devoid of ganglionic differentiation. Rosette

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*).

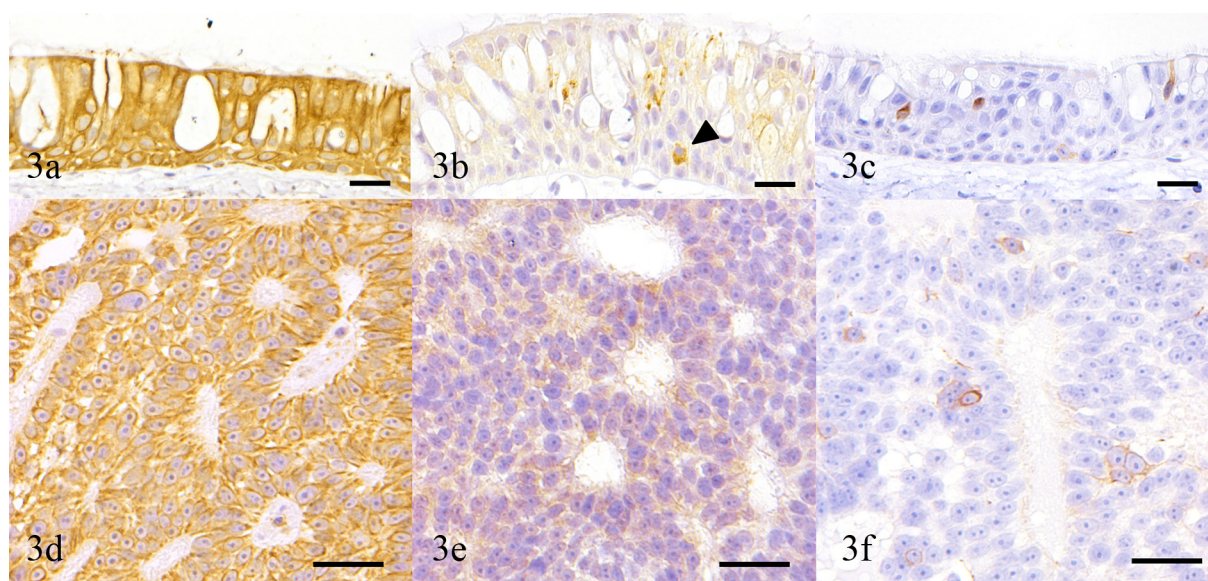


Fig. 3. Immunohistochemical findings of normal mucosal epithelium and neoplastic cells. (a) Normal mucosal epithelium is strongly positive for cytokeratin (CK). Scale bar=20 μ m. (b) Neuron-specific enolase (NSE)-positive cells are observed in the normal mucosal epithelium (arrowhead). Scale bar=20 μ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

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.

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