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NOTE

Pathology

Basal cell adenocarcinoma on bulbar conjunctiva of third eyelid in a dog

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ABSTRACT. An 8-year-old castrated Toy poodle presented with swelling and proptosis of the right third eyelid caused by an exophytic mass on the bulbar surface. Histologically, the mass was composed of stratified neoplastic basaloid cells, arranged in nests and interconnecting islands, which were mixed with tubular structures. Immunohistochemically, the basaloid cells were positive for p63 and cytokeratin (CK) 14, and the inner epithelial cells of the tubular structures were positive for CK7, CK8, and CK19. According to these findings, the mass was diagnosed as a basal cell adenocarcinoma. Although basal cell adenocarcinoma is rare in animals, it should be included in the list of differential diagnoses for superficial tumors of bulbar conjunctiva of third eyelid in dogs.

KEY WORDS: basal cell adenocarcinoma, conjunctiva, dog, immunohistochemistry

The conjunctiva is divided into the bulbar and palpebral conjunctivae, which cover the bulbar and palpebral surfaces, respectively. It is composed of the substantia propria covered by stratified squamous epithelium [20, 24]. The conjunctiva completely covers the third eyelid. The conjunctival epithelium of the third eyelid invaginates into the stroma of the third eyelid and forms the ducts of the gland of third eyelid.

Conjunctival tumors are relatively rare in dogs, but various types of benign and malignant canine conjunctival tumors have been reported, including cases of melanoma and melanocytoma [5], hemangiosarcoma and hemangioma [19], transmissible venereal tumors [15], mast cell tumors [8], lymphoma [14], angiokeratoma [2], and papilloma [25]. Conjunctival malignant epithelial tumors are extremely rare in dogs, and all of the reported cases involved squamous cell carcinoma (SCC) [13] or basal cell carcinoma (BCC) [21].

Basal cell adenocarcinoma (BCAC) is a malignant tumor, which is characterized by various types of proliferating basal and myoepithelial cells arranged in nests and ductal structures [9]. In humans, BCAC is classified as low malignancy, and it usually occurs in the main salivary glands, especially in the parotid gland [9]. Although such cases are uncommon, BCAC can occur as a free superficial neoplasm that originates from a minor salivary gland in the oral cavity or upper respiratory tract [10, 18]. In animals, BCAC is considered to be a rare neoplasm, and there have only been four studies about such cases, which included salivary gland tumors in two dogs and a cat [26], tumors of the third eyelid gland in dogs [16] and a bear [23], and a tumor of the parotid salivary gland in a big-eared opossum [3].

Conjunctival BCAC has not been described previously in veterinary medicine. In this report, we describe a case of primary BCAC, which occurred as a superficial tumor on the bulbar conjunctiva of the third eyelid in a dog.

An 8-year-old, castrated Toy poodle presented with swelling and proptosis caused by a mass on the right third eyelid and was treated with antibiotic and corticosteroidal eye ointment at a primary animal hospital for about 2 months, but no improvement was seen. An excisional biopsy examination was performed, and the specimen was submitted to a commercial diagnostic laboratory, which resulted in it being diagnosed as adenocarcinoma. The patient was referred to the Department of Veterinary Ophthalmology of Rakuno Gakuen University Animal Medical Center to undergo third eyelid extraction. Ophthalmic examinations did not reveal any abnormalities in either eyes or of the left third eyelid. The right third eyelid was slightly swollen, but the presence/absence of the mass could not be confirmed grossly because a restorative procedure involving a pocket-creating method had been conducted at the primary animal hospital. No abnormalities were found during other preoperative exanimations. Enucleation of the right third eyelid was performed, and the excised tissue was submitted for the histopathological examination.

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Grossly, a soft and whitish cauliflower-like mass, which measured approximately 0.6 × 1.0 × 0.3 cm in size, was located on the central area of the bulbar surface of the third eyelid (Fig. 1).

The tissue sample was fixed in 10% neutral-buffered formalin, processed routinely, and embedded in paraffin wax. Sections (thickness: 4 μm) were then stained with hematoxylin and eosin (HE) or periodic acid Schiff (PAS), before being subjected to light microscopic examinations. Immunohistochemical examinations were carried out using the avidin-biotin-peroxidase complex procedure (Vectastain Elite ABC kit; Vector Laboratories, Burlingame, CA, U.S.A.). The following primary antibodies were used: mouse monoclonal antibodies specific for human pan-cytokeratin (CK) (AE1/AE3; Nichirei, Tokyo, Japan; prediluted), porcine vimentin (V9; Dako, Glostrup, Denmark; prediluted), human p63 (MA1-21871; Thermo Fisher Scientific, Fremont, CA, U.S.A.; diluted 1:50), human CK14 (LL002; AbD serotec, Oxford, U.K.; diluted 1:50), human Ki-67 (MIB-1; Dako; diluted 1:50), human α-smooth muscle actin (αSMA; 1A4; Dako; diluted 1:100), human CK7 (RCK105; Abcam, Cambridge, U.K.; diluted 1:100), human CK8 (Ks8.7; Progen, Heidelberg, Germany; diluted 1:20), and human CK19 (BA17; Thermo Fisher Scientific; diluted 1:100). 3,-3′-diaminobenzidine was used as a chromogen, and the sections were counterstained with hematoxylin. In each immunohistochemical examination, the epidermis was used as a positive control for the detection of pan-CK, CK14, p63, and Ki-67, and the walls of blood vessels within the skin were used as a positive control for the detection of αSMA. Canine liver tissue was used as a positive control for the detection of CK7, CK8, and CK19.

Microscopically, the mass lacked a fibrous capsule, and it was partially covered by an epithelial layer. In addition, it displayed exophytic growth with a fibrovascular core, which extended from the superficial stroma of the third eyelid (Fig. 2). The gland of third eyelid was not involved in the mass (Fig. 3), however the ducts of the gland were diffusely dilated. The mass was composed of stratified neoplastic cells, which were arranged in nests and interconnecting islands, mixed with tubular structures (Fig. 4 and Fig. 4 inset). In the peripheral regions of the nests and islands, the nuclei of the neoplastic cells often demonstrated palisading along the interface of the fibrovascular stroma (Fig. 4 inset). At the margins of the mass, a gradual transition from the residual conjunctival basal layer to the neoplastic cells was evident. Mild invasion by the tumor nests was seen in the adjacent superficial stroma of the third eyelid. The neoplastic cells had relatively round to oval nuclei; were moderate in size; and exhibited a pale to granular chromatin pattern, small distinct nucleoli, moderate amounts of eosinophilic cytoplasm, and indistinct cell borders. The tubular structures were composed of peripheral neoplastic cells and inner epithelial cells, which had round to oval nuclei, were moderate in size, and displayed a granular chromatin pattern and small distinct nucleoli. The outermost epithelial cells that partially covered the mass did not exhibit cellular atypia. Instead, they had a similar histomorphological appearance to normal conjunctival epithelial cells. Scattered mitotic figures were observed (mean number in 10 high-power [magnification: x400] fields: 3.3). There was no necrosis or evidence of lymphatic or blood vessel invasion. PAS staining revealed mucus retention in the lumina of several tubular structures and thin basement membranes between the peripheral outer neoplastic cells and the stroma.

Immunohistochemically, the cytoplasm of the neoplastic cells was diffusely positive for pan-CK, and the nuclei of the neoplastic cells except for those of the inner epithelial cells of the tubular structures, were also diffusely positive for p63 (Fig. 5 and Fig. 5 inset). The cytoplasm of the neoplastic cells, but not that of the inner cells of the tubular structures, was positive for CK14 and partially positive for CK19, but negative for CK7, CK8, and αSMA. The inner epithelial cells of the tubular structures were positive for CK7 (Fig. 6), CK8, and CK19, but negative for p63, CK14 and αSMA. Vimentin and αSMA-positive cells were only observed in the stroma (Fig. 7). The outermost capsulated epithelial cells were positive for pan-CK, CK7, CK8, and CK19. The intratumoral Ki-67 nuclear index was 14.2%. The normal conjunctival epithelium and inner ductal epithelial cells of third eyelid gland were positive for pan-CK, CK7, and CK8, and the basal cell layers of these epithelia were positive for pan-CK, CK14, and p63. All of the inner ductal epithelial cells were positive for CK19, and some of the outer ductal epithelial cells and most of the acinar cells were also positive for CK19. The myoepithelial cells surrounding the acinar and intralobular ducts were positive for pan-CK, CK14, p63, and αSMA. In the positive control tissues, the epidermis expressed pan-CK, and CK14 and p63 were detected in the basal cell layer. Cells that exhibited nuclear positivity for Ki-67 were scattered throughout the basal cell layer. In the canine liver tissue, CK7, CK8, and CK19 were detected in the bile ducts, and the hepatocytes were positive for CK8.

Based on these histological and immunohistochemical findings, the conjunctival mass was diagnosed as a basal cell tumor with ductal differentiation; i.e., a BCAC.

In humans, BCAC is defined as a malignant tumor composed of various basal and myoepithelial cells, which form nests and ductal structures [9]. The mass in the present case was composed of neoplastic epithelial cell, which displayed peripheral palisading. The cells expressed CK14 and p63, but did not express αSMA. In addition, the expression of CK7, CK8, and CK19 was detected in the inner epithelial cell layers of the tubular structures. These findings suggested that the neoplastic epithelial cells differentiating into ductal epithelial cells, and that the conjunctival tumor was characterized by basal cell proliferation and ductal differentiation. Thus, even though the mass occurred at an unusual superficial site, it was diagnosed as a BCAC.

In dogs, although conjunctival tumor are relatively rare, various types of malignant and benign conjunctival tumors have been reported. Among canine conjunctival tumors, malignant epithelial tumors are uncommon, all of the reported cases involved SCC or BCC [13, 21]. The incidence rates of epithelial tumors differ among animal species, and the following types of conjunctival malignant epithelial tumors have been seen in animals: SCC and BCC in horses [1, 11], SCC in cows [22], and SCC and conjunctival mucoepidermoid carcinoma (conjunctival surface adenocarcinoma) in cats [4, 5]. There have only been four reports about BCAC in animals. The first report described the occurrence of the BCAC in the salivary glands of two dogs and a cat [26]. Two other reports described cases in which BCAC arose in the gland of the third eyelid in dogs [16] and or a bear [23]. Finally, a parotid salivary gland BCAC has recently been reported in a big-eared opossum [3].

Immunohistochemical analysis of the neoplastic cells in the current case demonstrated that the conjunctival BCAC was

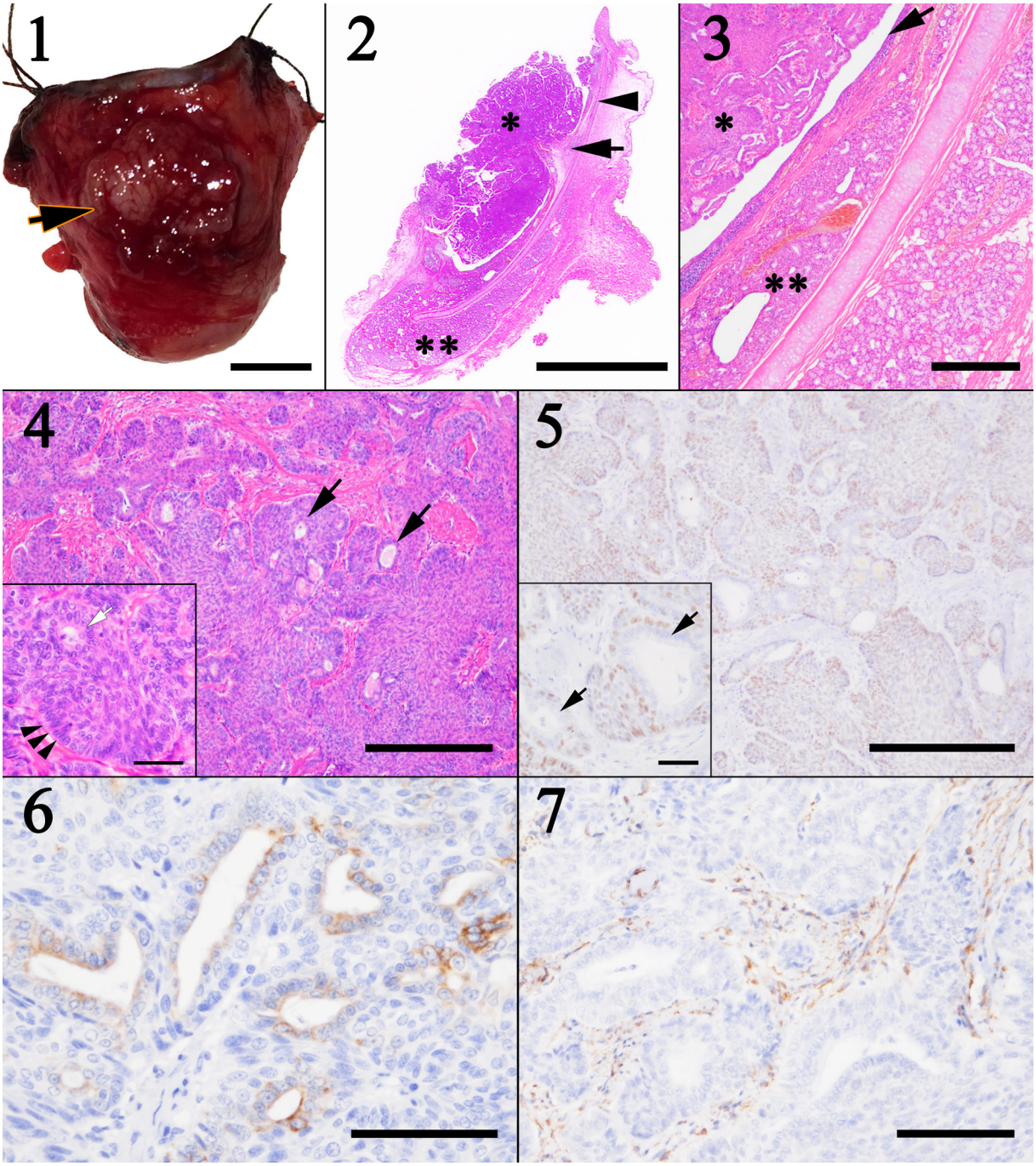

Fig. 1. Gross image of the right third eyelid after the extraction procedure. An exophytic cauliflower-like grown mass is present on the central area of bulbar conjunctiva (arrow). Bar=5 mm.

Fig. 2. Histological image of the sagittal section of the conjunctival mass and the right third eyelid. The mass (*) is located adjacent the bulbar conjunctiva of the third eyelid with a fibrous core (arrow). Arrowhead: cartilage of the third eyelid. HE. Bar=5 mm.

Fig. 3. Histological image of the conjunctival mass (*) and the right third eyelid. The gland of the third eyelid (**) is not involved in the tumor growth. Arrow: bulbar conjunctiva. HE. Bar=500 μm.

Fig. 4. Histological image of the conjunctival mass. The basaloid cells form nests and interconnecting islands associating with scattered tubular structures (arrows). HE. Bar=200 μm. (Inset) The nuclei of the neoplastic basaloid cells exhibit palisading (arrowheads). The tubular structures (white arrow) are surrounded by neoplastic basaloid cells. HE. Bar=30 μm.

Fig. 5. The nuclei of the neoplastic basaloid cells express p63, except for the inner epithelial cells of the tubular structures. Immunostaining of p63 counterstained with hematoxylin. Bar=100 μm. (Inset) Note that no p63 expression of the inner epithelial cells of the tubular structure (arrows). Immunostaining of p63 counterstained with hematoxylin. Bar=30 μm.

Fig. 6. The inner epithelial cells of the tubular structures express CK7. Immunostaining of CK7 counterstained with hematoxylin. Bar=50 μm.

Fig. 7. The αSMA express cells are only found in the stroma. Immunostaining of αSMA counterstained with hematoxylin. Bar=100 μm.

composed of cells that had not undergone myoepithelial differentiation. In human BCAC of the salivary gland, in addition to basal cell markers, such as CK14 and p63, the neoplastic cells often express αSMA, which indicates that they have differentiated into myoepithelial cells. However, limited expression of αSMA has been documented in human salivary and lacrimal gland BCAC [6, 12]. Among the reported canine and feline cases of salivary gland BCAC, moderate αSMA expression was observed in one canine case, but αSMA expression was not detected in the other canine case or the feline case [26]. The canine and ursine cases of BCAC of the third eyelid gland were also negative for αSMA [16, 23]. The differences in BCAC αSMA expression among animal cases considered to reflect the extent to which the neoplastic cells have differentiated into myoepithelial cells. Thus, the present case was characterized as a canine case of BCAC composed of neoplastic basaloid cells without myoepithelial differentiation.

In the current case, a transition from the conjunctival basal cells to the neoplastic basaloid cells was noted at the margins of the conjunctival tumor during the histological examination. In addition, the basal cells exhibited ductal differentiation, and the superficial stroma of the third eyelid was shown to be involved in the growth. Furthermore, no relationship between the tumor and the gland of the third eyelid was observed, but ductal dilation was apparent. These findings suggested that the source of the neoplastic cells in the present case is basal cells, which had the potential to differentiate into ductal epithelial cells, i.e., the basal cells located in the ductal opening region of the third eyelid gland [20]. A similar tumor origin had been suggested in feline conjunctival mucoepidermoid carcinoma (conjunctival surface adenocarcinoma) [4, 5]. It is considered that the feline tumor derives from the glandular duct epithelial cell, and extends out of the duct to form mass on the conjunctival surface.

In humans, BCAC often have a similar cytological and histomorphological appearance to the benign type of BCAC, which is known as basal cell adenoma (BCA) [7]. Although it is sometimes difficult to differentiate between malignant and benign tumors, attempts to differentiate between them can be made based on histological and immunohistochemical findings. The most important histological evidence of malignancy is local invasion, and the presence of 2 to 3 mitotic figures per 10 high-power fields is also an indicator of malignancy [7]. Regarding the Ki-67 proliferation index, a Ki-67 of >5% can be used as a threshold for differentiating between BCAC and BCA [17]. In the current case, the local invasion by the neoplastic cells was detected, and the mean number of mitotic figures per 10 high-power fields was 3.3. In addition, the tumor's Ki-67 index was about 3 times higher than the cut-off value for human BCAC. These histological and immunohistological findings indicated that the present case could be classified as a malignant BCA. In human BCAC of the salivary gland, local invasive growth and recurrence are occasionally seen, but distal metastasis and disease-related mortality are rare; therefore, BCAC is considered to be of low malignancy [7, 9]. In animals, no signs of metastasis were detected in previous cases of salivary gland BCAC (involving two dogs and a cat), and no recurrence or metastasis was observed in canine cases of third eyelid gland BCAC. According to these clinical findings, the authors concluded that BCAC are of low malignancy [16, 26]. In our case, the patient did not exhibit any signs of recurrence or distal metastasis during the 4 months after the extraction of the affected third eyelid. According to its clinical findings, the present case of BCAC on bulbar conjunctiva of third eyelid was presumed to be of low malignancy. However, the presence of a moderately high Ki-67 index suggested that the current case requires further monitoring.

This report described a case of BCAC on bulbar conjunctiva of third eyelid in a dog. Although BCAC is a rare neoplasm in animals, it should be included in the list of differential diagnoses for superficial tumors, especially those that arise on conjunctiva of third eyelid, in dogs.

REFERENCES

1. Baril, C. 1973. Basal cell tumour of third eyelid in a horse. *Can. Vet. J.* **14**: 66–67. [Medline]
2. Buyukmihci, N. and Stannard, A. A. 1981. Canine conjunctival angiokeratomas. *J. Am. Vet. Med. Assoc.* **178**: 1279–1282. [Medline]
3. Díaz-Delgado, J., Coimbra, A. A. C., Dos Santos-Cirqueira, C., Sanches, T. C., Guerra, J. M., de Oliveira, A. S., Di Loretto, C., Zwarg, T., Ressio, R., Rivas, L., Sansone, M., Nagamori, F. O., Kanamura, C., Gonçalves, P. S., Fernandes, N. C. C. A., Groch, K. R. and Catão-Dias, J. L. 2018. Parotid salivary gland basal cell adenocarcinoma in a Big-eared Opossum (Didelphis aurita). *J. Comp. Pathol.* **159**: 21–25. [Medline] [CrossRef]
4. Dubielzig, R. R. 2017. Tumors of the eye. pp. 892–922. *In*: Tumors in Domestic Animals, 5th ed. (Meuten, D. J. ed.), John Wiley & Sons, Inc., Hoboken.
5. Dubielzig, R. R., Kertring, K. L., McLallen, G. J. and Albert, D. M. 2010. Diseases of eyelids and conjunctiva. pp. 143–199. *In*: Veterinary Ocular Pathology: A Comparative Review, Sounders, Elsevier, St. Louis.
6. Ellis, G. 2005. Basal cell adenocarcinoma. pp. 230–231. *In*: Pathology and Genetics of Head and Neck Tumors (Barnes, L., Eveson, J. W., Reichart, P. and Sidransky, D. eds.), IARC Press, Lyon.
7. Ellis, G. L. and Auclair, P. L. 2008. Malignant epithelial neoplasms. pp. 173–438. *In*: Tumor of the Salivary Gland, AFIP Atlas of Tumor Pathology Series 4, Fascicle 9, Armed Forces Institute of Pathology, Washington, D.C.
8. Fife, M., Blocker, T., Fife, T., Dubielzig, R. R. and Dunn, K. 2011. Canine conjunctival mast cell tumors: a retrospective study. *Vet. Ophthalmol.* **14**: 153–160. [Medline] [CrossRef]
9. Fonseca, I., Gnepp, D. R., Seethala, R., Simpson, R. H. W., Vielh, P. and Williams, M. D. 2016. Basal cell adenocarcinoma. pp. 169–170. *In*: WHO Classification of Head and Neck Tumours, 4th ed. (EI-Naggar, A. K., Chan, J. K. C., Grandis, J. R., Takata, T. and Slootweg, P. J. eds.), IARC Press, Lyon.
10. Fonseca, I. and Soares, J. 1996. Basal cell adenocarcinoma of minor salivary and seromucous glands of the head and neck region. *Semin. Diagn. Pathol.* **13**: 128–137. [Medline]
11. Gelatt, K. N., Myers, V. S. Jr., Perman, V. and Jessen, C. 1974. Conjunctival squamous cell carcinoma in the horse. *J. Am. Vet. Med. Assoc.* **165**: 617–620. [Medline]
12. Khalil, M. and Arthurs, B. 2000. Basal cell adenocarcinoma of the lacrimal gland. *Ophthalmology* **107**: 164–168. [Medline] [CrossRef]
13. Lavach, J. D. and Snyder, S. P. 1984. Squamous cell carcinoma of the third eyelid in a dog. *J. Am. Vet. Med. Assoc.* **184**: 975–976. [Medline]
14. McCowan, C., Malcolm, J., Hurn, S., O'Reilly, A., Hardman, C. and Stanley, R. 2014. Conjunctival lymphoma: immunophenotype and outcome in

five dogs and three cats. *Vet. Ophthalmol.* **17**: 351–357. [Medline] [CrossRef]
15. Milo, J. and Snead, E. 2014. A case of ocular canine transmissible venereal tumor. *Can. Vet. J.* **55**: 1245–1249. [Medline]
16. Miyazaki, A., Yonemaru, K., Hirata, A., Yanai, T. and Sakai, H. 2015. Histopathological and immunohistochemical features of atypical epithelial tumours of the gland of the third eyelid in seven dogs. *J. Comp. Pathol.* **152**: 299–303. [Medline] [CrossRef]
17. Nagao, T., Sugano, I., Ishida, Y., Hasegawa, M., Matsuzaki, O., Konno, A., Kondo, Y. and Nagao, K. 1998. Basal cell adenocarcinoma of the salivary glands: comparison with basal cell adenoma through assessment of cell proliferation, apoptosis, and expression of p53 and bcl-2. *Cancer* **82**: 439–447. [Medline] [CrossRef]
18. Parashar, P., Baron, E., Papadimitriou, J. C., Ord, R. A. and Nikitakis, N. G. 2007. Basal cell adenocarcinoma of the oral minor salivary glands: review of the literature and presentation of two cases. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **103**: 77–84. [Medline] [CrossRef]
19. Pirie, C. G., Knollinger, A. M., Thomas, C. B. and Dubielzig, R. R. 2006. Canine conjunctival hemangioma and hemangiosarcoma: a retrospective evaluation of 108 cases (1989−2004). *Vet. Ophthalmol.* **9**: 215–226. [Medline] [CrossRef]
20. Prince, J. H., Diesem, C. D., Eglitis, I. and Ruskell, G. L. 1960. The dog. pp. 65–98. *In*: Anatomy and Histology of the Eye and Orbit in DOMESTIC ANIMALS, Charles C. Thomas, Springfield.
21. Rodriguez Galarza, R. M., Shrader, S. M., Koehler, J. W. and Abarca, E. 2016. A case of basal cell carcinoma of the nictitating membrane in a dog. *Clin. Case Rep.* **4**: 1161–1167. [Medline] [CrossRef]
22. Russell, W. O. and Loquvam, G. S. 1951. Squamous carcinoma of the conjunctiva and cornea of bovines ("cancer eye" of cattle). *Am. J. Pathol.* **27**: 727–728. [Medline]
23. Sakai, H., Goto, M. and Komatsu, T. 2017. Basal cell adenocarcinoma in the gland of the third eyelid of a brown bear (Ursus arctos). *J. Vet. Med. Sci.* **79**: 1348–1351. [Medline] [CrossRef]
24. Samuelson, D. A. 2013. Ophthalmic anatomy. pp. 39–170. *In*: Veterinary Ophthalmology, Vol. I, 5th ed. (Gelatt, K. N., Gilger, B. C. and Kern, T. J. eds.), John Wiley & Sons, Inc., Hoboken.
25. Sansom, J., Barnett, K. C., Blunden, A. S., Smith, K. C., Turner, S. and Waters, L. 1996. Canine conjunctival papilloma: a review of five cases. *J. Small Anim. Pract.* **37**: 84–86. [Medline] [CrossRef]
26. Sozmen, M., Brown, P. J. and Eveson, J. W. 2003. Salivary gland basal cell adenocarcinoma: a report of cases in a cat and two dogs. *J. Vet. Med. A Physiol. Pathol. Clin. Med.* **50**: 399–401. [Medline] [CrossRef]