The Journal of Veterinary Medical Science

NOTE

Pathology

Pleomorphic iridociliary adenocarcinoma with metastasis to the cervical lymph node in a chinchilla (*Chinchilla lanigera*)

Kengo UEDA[1)], Akiko UEDA[1)] and Kiyokazu OZAKI[2)]*

[1)]Vogel Animal Hospital, Kobe, Hyogo 657-0025, Japan
[2)]Laboratory of Pathology, Setsunan University, Osaka 573-0101, Japan

J. Vet. Med. Sci.
81(2): 193–196, 2019
doi: 10.1292/jvms.18-0517

Received: 28 August 2018
Accepted: 3 December 2018
Published online in J-STAGE: 14 December 2018

ABSTRACT. A tumor had formed in the right eye of a 14-year-old male chinchilla. The black-and-white-colored tumor occupied the entire eye except for the lens and had invaded extensively inside the orbit. Histologically, round, spindle- to polygonal-shaped tumor cells had proliferated in a solid-sheet arrangement. The tumor cells exhibited polymorphic nuclei ranging from round- to polygonal-shaped, as well as abundant cytoplasm, which occasionally contained melanin granules. In some areas, several cells were surrounded by the basal lamina. Additionally, the tumor showed cervical lymph-node metastasis. Upon immunostaining, the tumor cells were positive for epithelial markers (cytokeratin AE1/AE3, 8/18, and 20), S100, and vimentin. Consequently, we diagnosed primary pleomorphic iridociliary adenocarcinoma with lymph-node metastasis. This is the first report of iridociliary adenocarcinoma in chinchillas.

KEY WORDS: *Chinchilla lanigera*, iridociliary adenocarcinoma, lymph-node metastasis

Chinchillas have a lifespan of approximately 10 years, and although this is longer than that of other rodents, such as rats, mice, and hamsters, there have been few reports of tumors in chinchillas [6, 10–12, 14, 15]. Melanomas, schwannomas, and ciliary adenocarcinomas are extremely rare ocular tumors in mice and rats, as indicated in the National Toxicology Program database and in "New Toxicologic Histopathology" [7, 8]. Melanin-containing intraocular tumors include melanocytic and iridociliary tumors [2]. Differentiation between melanocytic and iridociliary tumors is relatively easy in well-differentiated tumors. However, it is usually difficult to differentiate melanocytic and iridociliary tumors when the tumor cells have few characteristic features. Cytokeratin immunostaining and analysis of basal lamina formation are crucial for differential diagnosis.

To our knowledge, there has been no previous report of ocular tumors in chinchillas. Here, we report a case of a pleomorphic iridociliary adenocarcinoma with metastasis to the cervical lymph node—which was difficult to differentiate from a melanoma—in a chinchilla.

A 14-year-old male chinchilla was brought to our clinic with chief complaints of right-eye rupture and eye pain. In addition to the right eye being enlarged and partially ruptured, the surrounding area was severely swollen, causing a mild subcutaneous swelling reaching the neck area (Fig. 1A). Blood tests and simple radiographic examination showed mild anemia and osteolysis in the right zygomatic bone. The right eye was enlarged and effaced by a black- to white-colored tumor. A lesion had infiltrated over the sclera, invading areas outside the eye and filling the orbit. Because the extent of the lesion in the right orbit rendered total removal difficult, enucleation of the eye and partial orbital exenteration were performed. The macroscopic mass, including the eye, was fixed in 10% neutral buffered formalin and embedded in paraffin. The paraffin sections were sliced and stained with hematoxylin and eosin. A periodic acid–methenamine–silver (PAM) stain was used for detecting the basement membrane. Immunohistochemical staining was performed by the labelled-polymer method using N-Histofine MAX PO (M or R) (Nichirei Biosciences, Tokyo, Japan) and the following primary antibodies: anti-MelanA (1:50; mouse monoclonal antibody; DAKO, Glostrup, Denmark); anti-cow S100 (1:320; rabbit polyclonal antibody; DAKO); anti-cytokeratin (CK) AE1/AE3 (1:400; mouse monoclonal antibody; DAKO); anti-vimentin (1:400; mouse monoclonal antibody; DAKO); anti-type IV collagen (1:200; rabbit polyclonal antibody; LSL, Tokyo, Japan); anti-CK8/18 (1:50; mouse monoclonal antibody; Novocastra, Berlin, Germany); anti-CK20 (1:20; mouse monoclonal antibody; DAKO); anti-desmin (ready for use; mouse monoclonal antibody; Nichirei); and anti-smooth muscle actin (1:40; mouse monoclonal antibody; DAKO) [4]. As a negative control, mouse or rabbit isotype immunoglobulin diluted to the same concentration was substituted for the primary antibody.

Histologically, the eye had been replaced by the tumor, with an almost total disappearance of normal ocular structures except for the lens (Fig. 1B). The tumor had grown outside the sclera and involved the muscles around the eye, causing extensive necrosis. Round,

*Correspondence to: Ozaki, K.: ozaki@pharm.setsunan.ac.jp

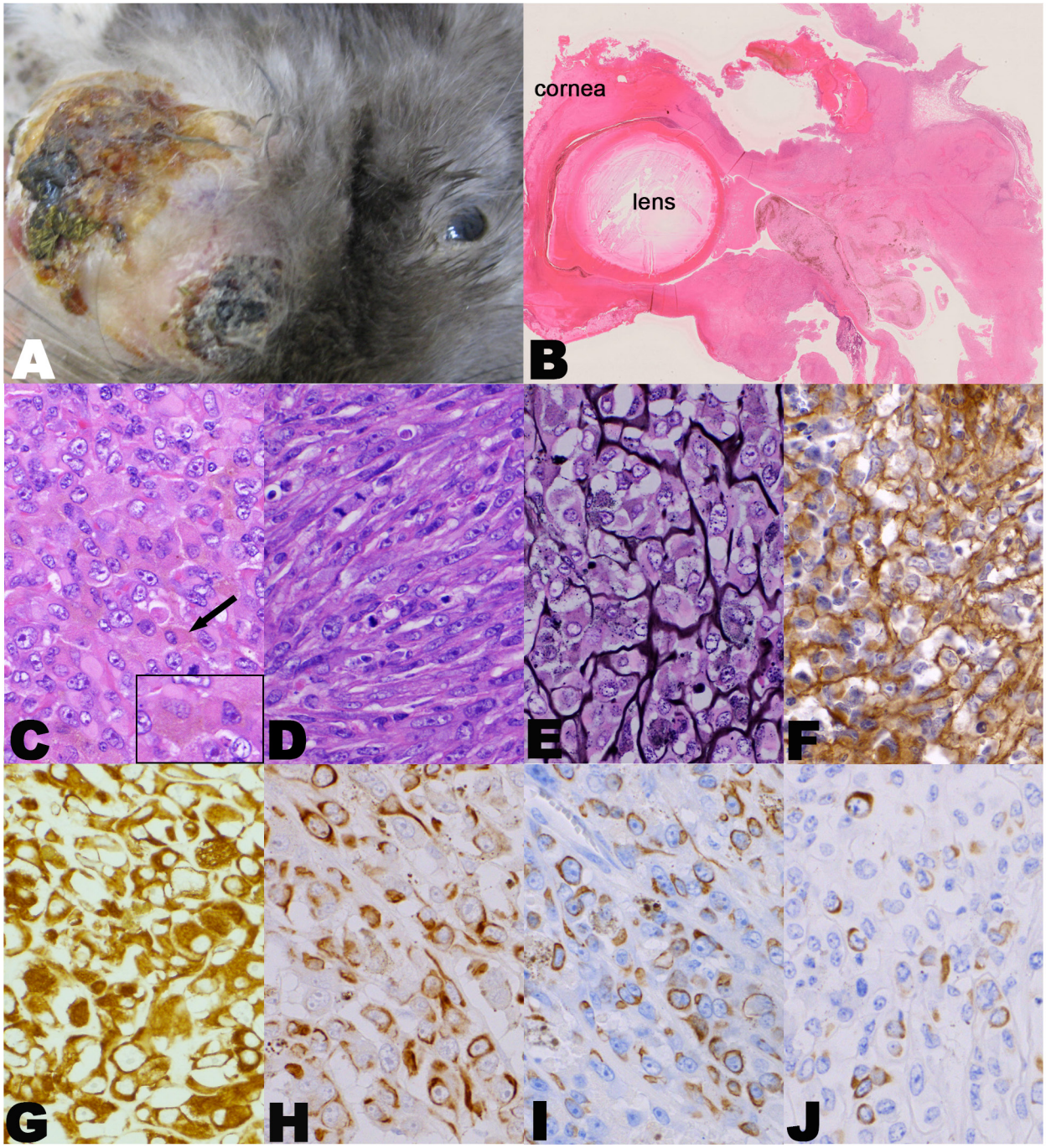

Fig. 1. (A) A tumor accompanied by ulceration and bleeding in the right eye, as well as severe swelling around the right eye. (B) The tumor occupied the entire eye except for the lens and had invaded the surrounding tissue. (C) The tumor cells exhibited clear polymorphism and abundant cytoplasm, which occasionally contained melanin granules (arrow). Inset,small melanin granules in tumor cells. (D) A region primarily consisting of spindle-shaped cells. No melanin granules were visible in this area. (E) Argyrophilic fibers surrounding one to several cells. (F) Basal membrane cells positive for type IV collagen, surrounding one or more cells. (G) All tumor cells were positive for vimentin. (H) Many cells were positive for cytokeratin AE1/AE3. (I) Numerous tumor cells were positive for cytokeratin 8/18 (J). Some tumor cells were positive for cytokeratin 20. (B–D) Hematoxylin–eosin staining; (E) Periodic acid–methenamine–silver staining; and (F–J) immunohistochemical staining.

spindle- to polygonal-shaped tumor cells had proliferated in a solid-sheet arrangement. The tumor cells exhibited polymorphic nuclei ranging from round- to polygonal-shaped (Fig. 1C and 1D). These cells showed distinct nucleoli, nuclei containing fine chromatin, and abundant cytoplasm, which occasionally contained melanin granules (Fig. 1C). The findings of PAM staining revealed a partial basal lamina surrounding the cell nest (Fig. 1E). This basal lamina was positive for type IV collagen (Fig. 1F).

Immunohistochemical findings demonstrated that the normal ciliary epithelial cells were positive for vimentin and S100 and negative for CK AE1/AE3, CK20, and CK8/18. The tumor cells were all positive for vimentin (Fig. 1G), 80% for S100 and CK AE1/AE3 (Fig. 1H), and 30% for CK20 (Fig. 1I) and CK8/18 (Fig. 1J). All tumor cells were negative for MelanA, smooth muscle actin, and desmin.

Postoperatively, the residual tumor tissue grew gradually, mainly in the right orbit, until the animal suddenly developed anorexia on postoperative day 45 and died. Autopsy findings revealed tumor growth outside the orbit and dissolution of the facial bones; they also revealed multiple focal necrosis in the liver and hematoma in the uterus. No gross lesions were observed in other organs. Histologically, the tumor had invaded extensively from the orbit to the surrounding tissue, and it also showed vascular and peripheral nerve invasion. Metastasis to the cervical lymph nodes was observed, although no distant metastasis (e.g., to the lungs) was visible.

This is the first report of a primary iridociliary adenocarcinoma of the eye in a 14-year-old chinchilla. The tumor had originated in the right eye, invaded intra- and extraorbital structures, and finally metastasized to the cervical lymph nodes. In veterinary medicine, iridociliary tumors are the second most common tumors in dogs after melanocytic tumors. They rarely occurs in cats [2] and have been reported in four cases of adenoma in rabbits [16] and two cases of adenoma in mice in the National Toxicology Program database and in "New Toxicologic Histopathology" [7]. However, they have never been reported in a chinchilla. In dogs, which have the highest number of reported cases, iridociliary tumors are categorized as adenomas or adenocarcinomas. Adenocarcinomas are infrequent, and highly malignant adenocarcinomas that invade surrounding tissues and metastasize are extremely rare. In fact, to date, there have only been two reported cases of such tumors, in a Labrador retriever and a Pembroke Welsh Corgi [2, 13, 17].

The presence of melanin granules was a characteristic feature of the tumor in the present case. Intraocular tumors that contain melanin granules include melanomas, melanocytomas, and iridociliary tumors [2]. It is easy to distinguish well-differentiated iridociliary tumors, even when they contain melanin granules. However, poorly differentiated polymorphic tumors are difficult to distinguish solely by hematoxylin–eosin staining, and accurate diagnosis requires immunostaining [2, 3]. Melanomas and melanocytomas are positive for melanocyte markers MelanA, vimentin, and S100 and negative for CK [1, 2, 5]. Iridociliary adenocarcinomas and adenomas are positive for vimentin and negative for CK AE1/AE3, although highly invasive adenocarcinomas become positive for CK AE1/AE3 [9]. Adenomas show CK20 expression, although it is reported to decrease with increasing tumor invasiveness [9]. The tumor in the present case was positive for AE1/AE3 and CK20, which are reported to be absent in melanocytic tumors. This tumor was also positive for CK8/18, which allowed us to exclude a diagnosis of melanocytic tumor.

Another characteristic feature of iridociliary tumors is basal lamina formation. Since there is no basement membrane in melanocyte tumors, this feature is very useful for differential diagnosis. Basal lamina formation is usually proven by using the PAS reaction [2, 3]. However, the PAS reaction is a histochemical method for demonstrating polysaccharides, and it cannot specifically stain the basement membrane. Therefore, in this study, we used PAM staining and type IV collagen immunostaining to more clearly and specifically recognize the basement membrane [17]. Since PAM staining can stain the basement membrane more delicately than the PAS reaction, it can clearly demonstrate even thin basement membranes [17]. However, a disadvantage of PAM staining is that it stains fibers other than the basement membrane. To compensate for this, we used type IV collagen, a component of the basement membrane, to prove that the PAM stain had indeed stained the basement membrane. In this case, the presence of a partial basal lamina was confirmed by PAM staining and type IV collagen immunostaining, which strongly suggested the presence of an iridociliary tumor.

Differentiation between an iridociliary adenoma and iridociliary adenocarcinoma is relatively simple. In dogs, tumors invading the area outside the sclera are diagnosed as adenocarcinomas, while those with intraocular invasion are diagnosed as adenomas [2]. The tumor in the present case was polymorphic, and it had invaded the region outside the sclera and metastasized. In addition, the tumor cells were positive for CK AE1/AE3, which occurs in highly invasive iridociliary adenocarcinomas [9]. These findings are consistent with those of a report of adenocarcinoma with metastasis in a dog [17]. The fact that metastatic adenocarcinomas exhibit the same histological characteristics in both dogs and chinchillas suggests that cellular polymorphism might be a marker of malignancy for this type of tumor.

In conclusion, the present case is the first report of iridociliary adenocarcinoma in chinchillas. Neoplasia should be considered as a differential diagnosis for a mass with rapid growth in this species.

REFERENCES

1. Busam, K. J., Kucukgöl, D., Sato, E., Frosina, D., Teruya-Feldstein, J. and Jungbluth, A. A. 2005. Immunohistochemical analysis of novel monoclonal antibody PNL2 and comparison with other melanocyte differentiation markers. *Am. J. Surg. Pathol.* **29**: 400–406. [Medline] [CrossRef]
2. Dubielzig, R. R., Ketring, K., McLellan, G. J. and Albert, D. M. 2010. The uvea. pp. 245–322. *In*: Veterinary Ocular Pathology: A Comparative Review (Dubielzig, R. R., Ketring, K., McLellan, G. J. and Albert, D. M. eds.), W. B. Saunders, New York.
3. Dubielzig, R. R., Steinberg, H., Garvin, H., Deehr, A. J. and Fischer, B. 1998. Iridociliary epithelial tumors in 100 dogs and 17 cats: a morphological study. *Vet. Ophthalmol.* **1**: 223–231. [Medline] [CrossRef]
4. Furukawa, S., Nagaike, M. and Ozaki, K. 2017. Databases for technical aspects of immunohistochemistry. *J. Toxicol. Pathol.* **30**: 79–107. [Medline] [CrossRef]
5. Giudice, C., Ceciliani, F., Rondena, M., Stefanello, D. and Grieco, V. 2010. Immunohistochemical investigation of PNL2 reactivity of canine

melanocytic neoplasms and comparison with Melan A. *J. Vet. Diagn. Invest.* **22**: 389–394. [Medline] [CrossRef]
6. Greenacre, C. B. 2004. Spontaneous tumors of small mammals. *Vet. Clin. North Am. Exot. Anim. Pract.* **7**: 627–651, vi vi. [Medline] [CrossRef]
7. Iwata, H., Maekawa, A., Tsuchitani, M. and Harada, T. 2017. Background data of mice. pp. 725–735. *In*: New Toxicologic Histopathology (Oishi, Y. ed.), Nishimurashoten, Tokyo.
8. Iwata, H., Maekawa, A., Tsuchitani, M. and Harada, T. 2017. Background data of rats. pp. 705–724. *In*: New Toxicologic Histopathology (Oishi, Y. ed.), Nishimurashoten, Tokyo.
9. Klosterman, E., Colitz, C. M., Chandler, H. L., Kusewitt, D. F., Saville, W. J. and Dubielzig, R. R. 2006. Immunohistochemical properties of ocular adenomas, adenocarcinomas and medulloepitheliomas. *Vet. Ophthalmol.* **9**: 387–394. [Medline] [CrossRef]
10. Lucena, R. B., Rissi, D. R., Queiroz, D. M. and Barros, C. S. 2012. Infiltrative gastric adenocarcinoma in a chinchilla (*Chinchilla lanigera*). *J. Vet. Diagn. Invest.* **24**: 797–800. [Medline] [CrossRef]
11. Newberne, P. M. 1953. Malignant lymphoma in a chinchilla. *Vet. Med.* **48**: 428–429.
12. Nobel, T. and Neumann, F. 1963. Carcinoma of the liver in a nutria (Myocastor coypus) and a chinchilla (Chinchilla laniger). *Refu Vet.* **20**: 161–162.
13. Okawauchi, M., Tsuboi, M., Nibe, K., Nagamine, E., Iwane, H. and Uchida, K. 2016. Iridociliary adenocarcinoma with oncocytic change in a dog. *J. Vet. Med. Sci.* **78**: 883–887. [Medline] [CrossRef]
14. Simova-Curd, S., Nitzl, D., Pospischil, A. and Hatt, J. M. 2008. Lumbar osteosarcoma in a chinchilla (*Chinchilla laniger*). *J. Small Anim. Pract.* **49**: 483–485. [Medline] [CrossRef]
15. Smith, J. L., Campbell-Ward, M., Else, R. W. and Johnston, P. E. 2010. Undifferentiated carcinoma of the salivary gland in a chinchilla (*Chinchilla lanigera*). *J. Vet. Diagn. Invest.* **22**: 152–155. [Medline] [CrossRef]
16. Swisher, S. D., Klein, H., Lennox, A. M., Reavill, D. R., Teixeira, L. B. C. and Shaw, G. C. 2018. Four cases of iridociliary tumors in domestic rabbits (*Oryctolagus Cuniculus*). *Vet. Ophthalmol.* **21**: 646–651 [CrossRef]. [Medline]
17. Zarfoss, M. K. and Dubielzig, R. R. 2007. Metastatic iridociliary adenocarcinoma in a labrador retriever. *Vet. Pathol.* **44**: 672–676. [Medline] [CrossRef]