



NEOPLASTIC DISEASE

Thymic Carcinoma with Cartilage Formation in a Dog

J. K. Chambers^{*}, Y. Yoshino^{*}, R. Fujiwara[†], R. Nishimura[‡],
H. Nakayama^{*} and K. Uchida^{*}

^{*} Department of Veterinary Pathology, [†] Veterinary Medical Center and [‡] Department of Veterinary Surgery, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo, Japan

Summary

An 11-year-old female Chihuahua exhibited respiratory distress and a computed tomography scan showed a large mass in the anterior thoracic cavity. During surgery, it was found that the mass was strongly adherent to surrounding tissue. A histopathological examination of a biopsy sample from the mass revealed proliferation of atypical epithelial cells and cartilage formation admixed with mature lymphocytes. Immunohistochemically, the tumour cells, as well as the normal canine thymic epithelial cells, were positive for pan-cytokeratin (CK), CK5/6, CK19, p63 and bone morphogenetic protein (BMP) 6. Foci of cartilage tissue were formed in association with the neoplastic epithelial tissue. In the normal canine thymus, the subcapsular epithelial cells are positive for both CK19 and BMP6. These findings indicate that the cartilage element within the tumour developed from CK19-positive neoplastic epithelial cells, which were derived from the thymic subcapsular epithelium. This case represents a novel variant of canine thymic epithelial tumour that exhibits cartilage differentiation.

© 2015 Elsevier Ltd. All rights reserved.

Keywords: cartilage; dog; thymic carcinoma; thymic epithelial cell

Tumours derived from the thymic epithelium are called thymomas or thymic carcinomas. In dogs, thymoma is the most common type of thymic tumour, followed by thymic lymphoma (Day, 1997). Thymic mesenchymal tumours are rare in animals, although canine cases of thymolipoma (Ramírez *et al.*, 2008) and thymofibroli-poma (Morini *et al.*, 2009; Tobias and Cullen, 2014) are reported. In people, sclerosing thymoma and lipofibroadenoma consist of both thymic epithelial cells and mesenchymal components and are considered to be extremely rare (Marx *et al.*, 2004). The purpose of this report is to describe a canine case of thymic carcinoma involving cartilage formation.

An 11-year-old female Chihuahua exhibited respiratory distress. Thoracic radiography and computed tomography (CT) examinations revealed a large mass (5 × 3.5 × 2.5 cm) within the anterior thoracic cavity (Fig. 1). Surgery was performed to excise the

tumour; however, severe pleural adhesion made it impossible to remove the entire mass. During radiographical and gross examinations, the tumour was confirmed to be located in the region of the thymus and metastatic lesions were not seen in the lungs or mediastinal lymph nodes. The prognosis for the dog was considered to be poor and no further treatment or clinical follow-up was performed.

A sample from the ventral region of the tumour was fixed in 10% neutral-buffered formalin for histopathological diagnosis. The tissue was processed routinely and embedded in paraffin wax. Sections (4 µm) were stained with haematoxylin and eosin (HE). Immunohistochemistry (IHC) was performed using the EnVision + System, a horseradish peroxidase-labelled polymer (Dako, Tokyo, Japan) and 3, 3'-diaminobenzidine tetrahydrochloride (DAB) as a chromogen. Paraffin wax-embedded normal thymic tissue (collected from a 2-year-old dog during a routine necropsy examination) was used as a positive control. The following primary antibodies were used:

Correspondence to: K. Uchida (e-mail: auchidak@mail.ecc.u-tokyo.ac.jp).

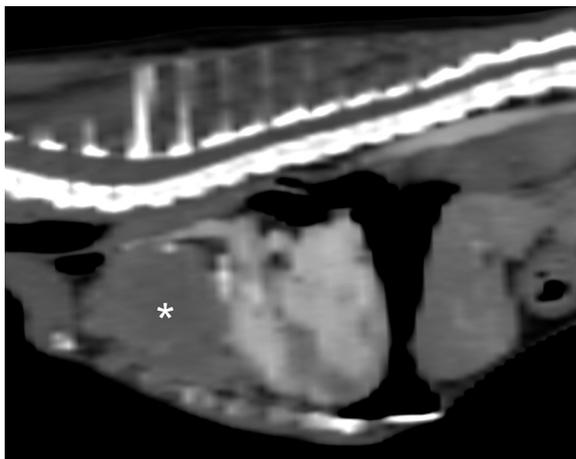


Fig. 1. CT image of the thoracic region. A large mass (asterisk) is located anterior to the heart.

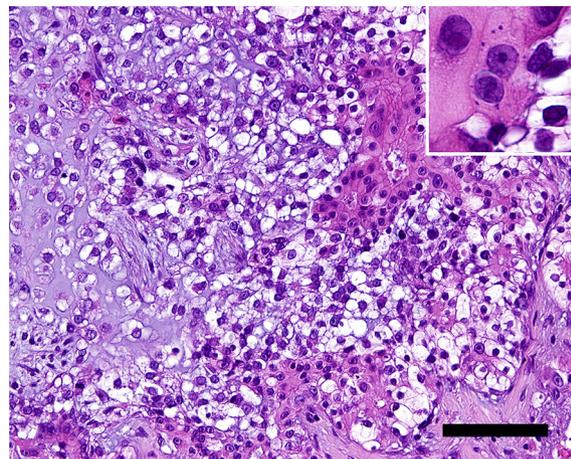


Fig. 2. The tumour is composed of epithelial cells with clear or eosinophilic cytoplasm and cartilage tissue. The cartilage tissue (left) is contiguous with the epithelial component (right) of the tumour. Inset shows keratohyalin granules in a tumour cell. HE. Bar, 50 μ m.

anti-pan-cytokeratin (CK) (clone AE1/AE3, ready to use; Dako), anti-CK5/6 (clone D5/16 B4, 1 in 100 dilution; Dako), anti-CK19 (clone B170, ready to use; Leica, Newcastle, UK), anti-bone morphogenetic protein (BMP) 6 (goat polyclonal, 1 in 50 dilution; Santa Cruz, Dallas, Texas, USA), anti-p63 (clone BC4A4, 1 in 100 dilution; Biocare Medical, Concord, California, USA), anti-CD3 (rabbit polyclonal, 1 in 50 dilution; Dako), anti-CD20 (rabbit polyclonal, 1 in 400 dilution; ThermoFisher Scientific, Fremont, California, USA), anti-thyroglobulin (rabbit polyclonal, ready to use; Dako) and anti-chromogranin A (rabbit polyclonal, 1 in 200 dilution; Yanaihara Institute, Shizuoka, Japan). A double-labelling immunofluorescence technique was also performed using Alexa Fluor 488-conjugated donkey anti-goat IgG (1 in 200 dilution; Invitrogen, Eugene, Oregon, USA) and Alexa Fluor 594-conjugated goat anti-mouse IgG (1 in 200 dilution; Life Technologies, Eugene, Oregon, USA) as secondary antibodies, HardSet™ mounting medium and 4', 6-diamidino-2-phenylindole (DAPI; Vectashield, Burlingame, California, USA) as nuclear counterstain.

Microscopically, the surgical biopsy sample comprised of neoplastic epithelial tissue with fibrous stroma and multifocal necrosis. Lymphocytes and a few mast cells were admixed with the neoplastic tissue. The neoplastic tissue consisted mainly of sheets of cells with occasional acinar-like structures and foci of cartilage (Fig. 2; Supplemental Fig. S1a, b). The tumour cells were round and had clear or eosinophilic cytoplasm. Keratohyalin granules were seen in the neoplastic cells (Fig. 2). Moderate anisocytosis, anisokaryosis and nuclear atypia were observed; however, mitotic figures were rare (mitotic index <1 per \times 400 field).

The immunohistochemical profiles of normal canine thymic epithelial cells are described in Supplemental Table S1 and shown in Supplemental Figs. S2a–e. In the normal canine thymus, pan-CK, CK19 and p63 were broadly expressed by thymic epithelial cells (in the subcapsular region, cortex and medulla), while CK5/6 expression was restricted to the epithelial cells in the thymic medulla. BMP6-expressing cells were mainly observed in the subcapsular region. The double-labelling immunofluorescence examination revealed that the CK19-positive subcapsular epithelial cells also expressed BMP6 (Supplemental Fig. S2f). The clear neoplastic cells were positive for pan-CK, CK19 and p63, while the eosinophilic neoplastic cells were positive for pan-CK, CK5/6 and CK19 (Figs. 3 and 4; Supplemental Figs. S3a–c). In addition, the clear cells, eosinophilic cells and the cells inside the cartilage lacuna (chondroid cells) were positive for pan-CK, CK19 and BMP6 (Figs. 3 and 4 and Supplemental Figs. S3c, d). The neoplastic cells were negative for thyroglobulin and chromogranin A. The neoplastic tissue contained similar numbers of CD3-positive T cells and CD20-positive B cells.

In the present case, the tumour tissue was composed of atypical epithelial cells with occasional cytoplasmic keratohyalin granules, indicating that the tumour was of thymic epithelial origin. In human medicine, CK19 and p63 are established markers of both neoplastic and normal thymic epithelial cells (Kuo, 2000; Dotto *et al.*, 2007). To our knowledge, the present study is the first to characterize the expression of CK19 and p63 in normal and neoplastic canine thymic epithelial cells

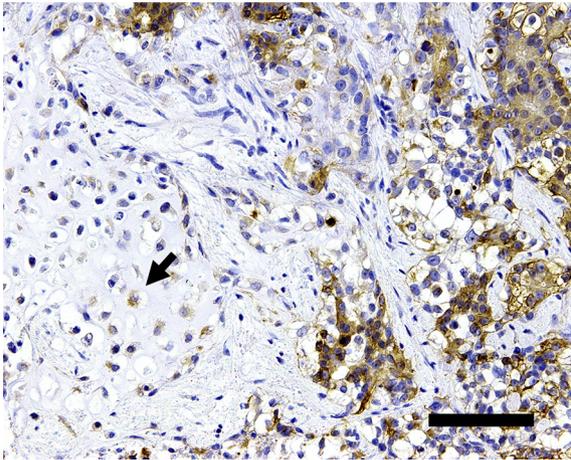


Fig. 3. Expression of CK within the cytoplasm of the tumour cells. The chondroid cells inside the lacuna are also positive for pan-CK (arrow). IHC. Bar, 50 μ m.

(Supplemental Figs. S2a, c). Based on the above findings, together with the poorly delineated margins and biological behaviour of the tumour (i.e. pleural adhesion), a diagnosis of thymic carcinoma was made.

Epithelial tumours containing cartilage and/or osteogenic elements are often encountered in mixed canine mammary gland tumours and human salivary gland pleomorphic adenomas. In these tumours, both the epithelial tumour cells and the chondroid cells (lacuna cells) express BMP6, indicating that the cartilage is formed in response to BMP6 expression by neoplastic epithelial cells (Kusafuka *et al.*, 1999; Tateyama *et al.*, 2001). Moreover, Tateyama *et al.* (2001) reported that resting myoepithelial cells normally express BMP6. However, it is yet to be elucidated whether the cartilage tissue in mixed canine

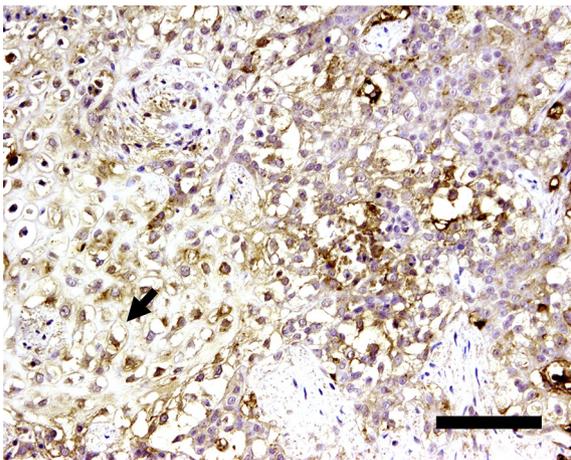


Fig. 4. Expression of BMP6 within the cytoplasm of the tumour cells. The chondroid cells inside the lacuna are also positive for BMP6 (arrow). IHC. Bar, 50 μ m.

mammary tumours is directly derived from myoepithelial cells or is taken up by myoepithelial cells from the surrounding stromal tissue. In the present study, BMP6 expression was detected in both the tumour cells and CK19-positive normal thymic epithelial cells, indicating that BMP6 might have played a role in the cartilage formation seen in the present case.

Cartilage and/or bone formation can occur in canine thyroid follicular cell tumours (Grubor and Haynes, 2005; Kobayashi *et al.*, 2014). Thyroid tumours sometimes arise from ectopic thyroid tissue around the heart (Capen, 2007; Almes *et al.*, 2008); however, a thyroid follicular cell tumour was ruled out in the present case because the tumour cells were positive for p63 and negative for thyroglobulin. Carcinoma showing thymus-like differentiation of the thyroid (CASTLE) is a rare type of tumour affecting the human thyroid gland (Reimann *et al.*, 2006). CASTLE tumour cells are positive for p63 and negative for thyroglobulin, indicating that such tumours arise from the ectopic thymic tissue found in the remnant pharyngeal pouches.

The thymus develops from the third and fourth pharyngeal pouches during embryogenesis (Suster and Rosai, 2007). The endodermal lining and the mesenchyme (derived from the neural crest) of the pharyngeal pouches give rise to the epithelial cells and supporting stroma of the thymus, respectively. At the same time, the cartilage tissue of the head and neck (i.e. hyoid cartilage) is produced from the pharyngeal pouch mesenchyme (Ruhin *et al.*, 2003; Crump *et al.*, 2004). Canine oral melanomas occasionally exhibit osteocartilaginous differentiation, which is considered to be a trait of neural crest-derived melanocytes (Sánchez *et al.*, 2007). In the present case, the cartilage component was either produced by the neoplastic cells from the surrounding mesenchyme or arose via neoplastic cell differentiation. The structural continuity of the epithelial and cartilage components, as well as the detection of pan-CK, CK19 and BMP6 expression in the chondroid cells, indicates that the cartilage tissue developed directly from the neoplastic thymic epithelial cells (Figs. 2 and 3). Also, the results of the immunohistochemical examination indicated that the tumour originated from the subcapsular CK19- and BMP6-positive thymic epithelial cells.

To the best of our knowledge, cartilage formation in a thymic epithelial tumour has never been reported before. The present report describes a rare variant of thymic epithelial tumour that should be added to the classification of canine neoplastic diseases.

Acknowledgments

This study was supported in part by JSPS KAKENHI grant number 26660236.

Conflict of Interest Statement

The authors declare that there are no potential conflicts of interest related to the research or the authorship and/or publication of this article.

Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jcpa.2015.11.001>.

References

- Almes KM, Heaney AM, Andrews GA (2008) Intracardiac ectopic thyroid carcinosarcoma in a dog. *Veterinary Pathology*, **45**, 500–504.
- Capen CC (2007) Thyroid gland. In: *Pathology of Domestic Animals*, 5th Edit., Vol. 3, MG Maxie, Ed., Elsevier Saunders, Philadelphia, pp. 379–407.
- Crump JG, Swartz ME, Kimmel CB (2004) An integrin-dependent role of pouch endoderm in hyoid cartilage development. *PLoS Biology*, **2**, e244.
- Day MJ (1997) Review of thymic pathology in 30 cats and 36 dogs. *Journal of Small Animal Practice*, **38**, 393–403.
- Dotto J, Pelosi G, Rosai J (2007) Expression of p63 in thymomas and normal thymus. *American Journal of Clinical Pathology*, **127**, 415–420.
- Grubor B, Haynes JS (2005) Thyroid carcinosarcoma in a dog. *Veterinary Pathology*, **42**, 84–87.
- Kobayashi R, Yamada N, Kitamori T, Kitamori F, Sato K *et al.* (2014) Follicular thyroid carcinoma characterized by abundant stromal components with chondroid and osseous metaplasia in a dog. *Journal of Veterinary Medical Science*, **76**, 1161–1164.
- Kuo TT (2000) Cytokeratin profiles of the thymus and thymomas: histogenetic correlations and proposal for a histological classification of thymomas. *Histopathology*, **36**, 403–414.
- Kusafuka K, Yamaguchi A, Kayano T, Takemura T (1999) Immunohistochemical localization of the bone morphogenetic protein-6 in salivary pleomorphic adenomas. *Pathology International*, **49**, 1023–1027.
- Marx A, Chen G, Strobel Ph, Kuo TT, Zhang PJ (2004) Tumours of the thymus. In: *World Health Organization Classification of Tumours of the Lung, Pleura Thymus and Heart*, WD Travis, E Brambilla, HK Muller-Hermelink, CC Harris, Eds., IARC Press, Lyon, p. 171.
- Morini M, Bettini G, Diana A, Sladari A, Casadio Tozzi A *et al.* (2009) Thymofibrolipoma in two dogs. *Journal of Comparative Pathology*, **141**, 74–77.
- Ramírez GA, Spattini G, Altimira J, García B, Vilafranca M (2008) Clinical and histopathological features of a thymolipoma in a dog. *Journal of Veterinary Diagnostic Investigation*, **20**, 360–364.
- Reimann JD, Dorfman DM, Nosé V (2006) Carcinoma showing thymus-like differentiation of the thyroid (CASTLE): a comparative study: evidence of thymic differentiation and solid cell nest origin. *American Journal of Surgical Pathology*, **30**, 994–1001.
- Rubin B, Creuzet S, Vincent C, Benouaiche L, Le Douarin NM *et al.* (2003) Patterning of the hyoid cartilage depends upon signals arising from the ventral foregut endoderm. *Developmental Dynamics*, **228**, 239–246.
- Sánchez J, Ramirez GA, Buendia AJ, Vilafranca M, Martinez CM *et al.* (2007) Immunohistochemical characterization and evaluation of prognostic factors in canine oral melanomas with osteocartilaginous differentiation. *Veterinary Pathology*, **44**, 676–682.
- Suster S, Rosai J (2007) Thymus. In: *Histology for Pathologists*, 3rd Edit., SE Mills, Ed., Lippincott Williams & Wilkins, Philadelphia, pp. 505–525.
- Tateyama S, Uchida K, Hidaka T, Hirao M, Yamaguchi R (2001) Expression of bone morphogenetic protein-6 (BMP-6) in myoepithelial cells in canine mammary gland tumors. *Veterinary Pathology*, **38**, 703–709.
- Tobias JR, Cullen JM (2014) Thymofibrolipoma in a Labrador retriever. *Veterinary Pathology*, **51**, 816–819.

[Received, August 12th, 2015]
 [Accepted, November 2nd, 2015]