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NEOPLASTIC DISEASE

Thymic Carcinoma with Cartilage Formation in a Dog

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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.

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 thymofibrolipoma (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:

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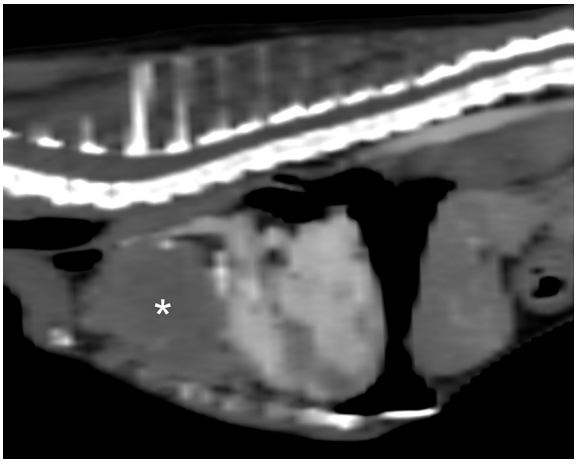

Fig. 1. CT image of the thoracic region. A large mass (asterisk) is located anterior to the heart.

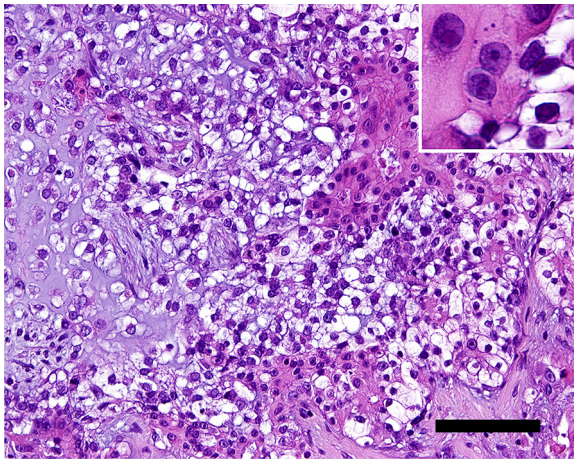

Fig. 2. The tumour is composed of epithelial cells with clear or eosinophilic cytoplasm and cartilage tissue. The cartilage tissue (left) is contiguous with the epithelial component (right) of the tumour. Inset shows keratohyalin granules in a tumour cell. HE. Bar, 50 μm.

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 × 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