Follicular Thyroid Carcinoma Characterized by Abundant Stromal Components with Chondroid and Osseous Metaplasia in a Dog

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ABSTRACT. A dog developed a cervical mass, and computed tomography verified a mass surrounding the trachea with some pulmonary masses. Histopathologically, the cervical mass was composed of malignant neoplastic cells showing follicular appearance which reacted positive for thyroglobulin on immunohistochemistry. A characteristic feature of the tumor was abundant and metaplastic stromal components. Anastomosed collagenous tissues connecting to capsule of the tumor were abundant in the stroma. In parts of the collagenous tissues, mature cartilages and bones were continuously formed. There was no cellular atypia or invasion in the components. We diagnosed this case as follicular thyroid carcinoma with metaplastic stroma. This is the first case report that characterizes stromal components with chondroid and osseous metaplasia in a canine thyroid carcinoma.
KEY WORDS: canine, chondroid metaplasia, follicular thyroid carcinoma, osseous metaplasia, tumor stroma

Thyroid carcinomas (follicular cell carcinomas) are diagnosed more frequently than adenomas in dogs and are classified into well-differentiated, poorly-differentiated and undifferentiated thyroid carcinomas [7]. Well-differentiated thyroid carcinomas are subdivided into follicular, compact, follicular-compact and papillary thyroid carcinomas on the basis of predominant histologic pattern, and the neoplastic cells form multinodular nests including hemorrhage and necrosis. It has been described that some canine thyroid carcinomas form bone in the stroma [4]. In addition, stromal chondroid and osseous metaplasia have also been reported [2]. However, there was limited information concerning the stromal components in the literatures. This is the first case report that characterizes abundant stromal components with chondroid and osseous metaplasia in a canine follicular thyroid carcinoma.

A 12-year-old, spayed female, mixed-breed dog with a cervical mass was taken to an animal hospital. Computed tomography revealed the mass surrounding the trachea with patchy regions of high density which was comparable to bone tissues (Fig. 1). Some pulmonary masses were also revealed, but had no high density regions. The cervical mass was resected by surgery and then fixed in 10% neutral formalin. Macroscopically, the mass was approximately 8 × 7 × 3 cm, solid and relatively well-demarcated and irregular

in shape. The cut surface was creamy-white and had partial sandy texture (Fig. 2). After surgery, the dog developed a cough, and decreasing thyroxine and increasing total cholesterol in the serum were elucidated. Serum thyroxine was controlled with a thyroid hormonal agent, and myxedema was not observed. The dog died of respiratory failure eight months after surgery, and necropsy was not done.

Samples from the formalin-fixed mass were embedded in paraffin, cut at 4 μm and stained with hematoxylin and eosin (HE), alcian blue and the Watanabe's method for reticulin fibers. Calcified solid samples were decalcified with 10% formic acid for several days before embedding. Immunohistochemical staining was performed using the immunoenzyme polymer method. The 4 μm sections were pretreated with citrate buffer (pH 6.0) in a microwave for 15 min at 95°C (for all antibodies except cytokeratin) or Proteinase K Ready-to-use (for cytokeratin; Dako, Glostrup, Denmark) for 3 min at room temperature. After the sections were treated with 3% hydrogen peroxide, blocking solution (Dako) was applied and incubated for 10 min at room temperature. The following primary antibodies were reacted at 4°C overnight; polyclonal rabbit anti-thyroglobulin (Dako; pre-diluted), polyclonal rabbit anti-calcitonin (Dako; 1:100), monoclonal mouse anti-synaptophysin (clone SY 38; PROGEN, Heidelberg, Germany; 1:50), polyclonal rabbit anti-cytokeratin wide spectrum screening (Dako; 1:100), monoclonal mouse anti-vimentin (clone V9; Dako; 1:100), monoclonal mouse anti-smooth muscle actin (α-SMA; clone 1A4; Dako; 1:200) and monoclonal mouse anti-proliferating cell nuclear antigen (PCNA; clone PC10; Dako; 1:200). Peroxidase-conjugated anti-mouse/rabbit IgG (Nichirei, Tokyo, Japan) was used as the secondary antibody for 30-min incubation at room temperature. The sections were color developed with DAB

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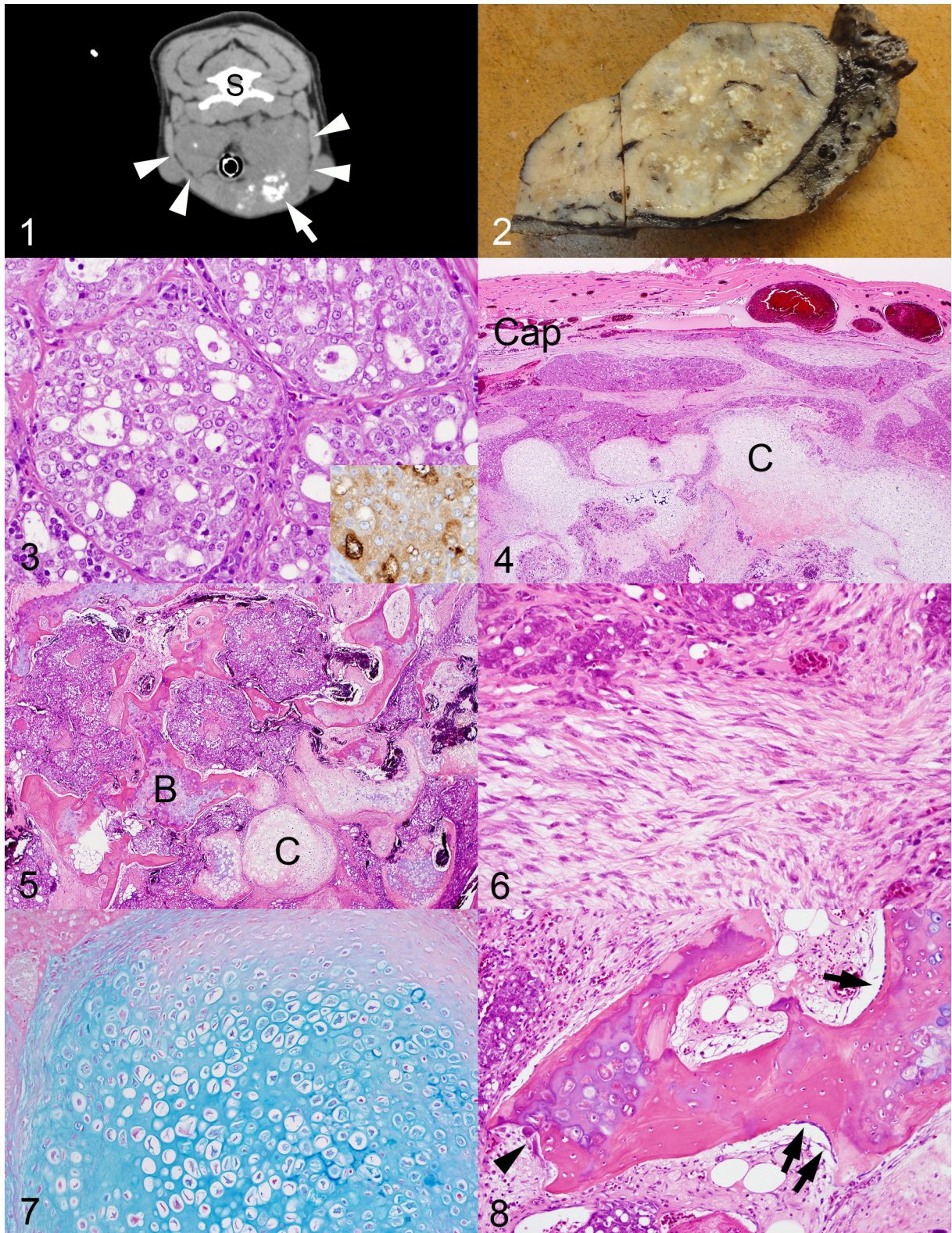
solution (Nichirei) and counterstained with hematoxylin. As the positive control, normal canine thyroid tissue was used.

Histopathologically, the cervical mass was encapsulated by connective tissue and divided into a large number of neoplastic nests, and no native thyroid tissues were observed in any sections. Focal necrosis, cholesterin clefts and lymphoplasmacytic infiltration were scattered. Two histologic patterns of the neoplastic cells were observed in the tumor. The first pattern showed follicular appearances, being absolutely predominant in the tumor. The neoplastic cells were cuboidal with round to oval and vesicular nuclei and amphophilic to eosinophilic cytoplasms, and these cells formed a number of miniature follicles, or cribriform or solid nests (Fig. 3). Mitotic figures were frequently seen (28 per 10 high-powered fields). The second pattern showed compact sheets resembling C cells. The neoplastic cells had small round nuclei and clear cytoplasm with obscure cell borders and formed small and solid nests or trabecular structures. By immunohistochemistry, the neoplastic cells in both areas were strongly positive for thyroglobulin (Fig. 3) and cytokeratin, while negative for calcitonin or synaptophysin. Moreover, a large number of the cells were positive for PCNA (55 positive cells per 100 tumor cells; 55%). These findings indicated that malignancy of the tumor and the neoplastic cells originated from thyroid follicular cells.

On the other hands, abundant mesenchymal tissues formed in the tumor stroma. In the stroma, collagenous tissues connecting capsule of the tumor formed anastomosed branch of collagen bundles across the neoplastic tissues (Fig. 4). Additionally, multifocal cartilage and bone were continuously formed from the collagenous tissues (Figs. 4 and 5). The collagenous component was composed of mature collagen fibers and interlaced with spindle cells with uniform elongate or oval nuclei (Fig. 6). Immunohistochemically, the spindle cells were positive for vimentin and negative for cytokaratin or thyroglobulin. A small portion of the cells positively stained with α-SMA or PCNA (16 positive cells per 100 spindle cells; 16%). In the chondral component, monomorphic chondrocytes that had single and crumpled nuclei in hyalinized cartilages were observed. The cartilage matrices were stained with pale to deep blue for alcian blue staining (Fig. 7) and were partially calcified. From the edge of the calcified cartilages, the osseous components were

formed, suggesting endochondral ossification. These osseous tissues were composed of lamellar bone matrix containing osteocytes within the lacunae and were partly fringed with osteoblasts which had pyknotic nucleus and flattened cytoplasm (Fig. 8). Some multinucleated osteoclasts were contacted with the border of the bone. No mitotic figure or atypia was observed in the fibroblasts, chondrocytes and osteoblasts at any sites. The cartilage and bone were thoroughly surrounded by neoplastic parenchyma, and there was no independent proliferation of the chondral or osseous component without fibrous tissue. In the cartilage and bone, necrosis of adjacent tissue associated with invasion was not observed. The stromal components were clearly separated from neoplastic parenchyma by basement membranes highlighted in Watanabe's method for reticulin fibers. The amount of stroma varied remarkably depending on the neoplastic area examined.

We diagnosed the case as follicular thyroid carcinoma with abundant stromal components based on the view that the stromal components underwent alteration that was not neoplastic, but metaplastic. The tumor was characterized by mixed features of follicular neoplastic cells and abundant mesenchymal components with osseous and chondroid metaplasia in the stroma. It has been reported bone or cartilage formation in the stroma of canine follicular carcinoma [2, 4]; however, details of the stromal components were not described particularly.

As differential diagnosis, thyroid carcinosarcoma (canine malignant mixed tumor of thyroid) which is characterized by malignant follicular and malignant mesenchymal components was included. In fact, at first, we suspected the thyroid carcinosarcoma due to superficial proliferation of the mesenchymal components in the present case. In published reports, sarcomatous components in carcinosarcoma showed various differentiation including fibrous, chondral, osseous and sometime vascular components [1, 3, 5, 6, 8]. The origin or clonality of the components remained unclear [7]. In these reports, malignancy of the sarcomatous components was demonstrated based on cellular atypia and high mitotic index of the mesenchymal cells. Clinically, each carcinomatous and sarcomatous component could metastasize to other organs, such as the lungs [5]. It was considered that the mesenchymal components in our case would not show

Fig. 1. Computed tomography image of the neck. The mass surrounding the trachea (arrowheads) is observed. There are patchy regions of high density (arrow) which is comparable to that of bone. S; spine.

Fig. 2. Cut surface of the cervical mass. The color is creamy-white, and white areas that have a sandy texture are scattered within the mass.

Fig. 3. Neoplastic follicular cells form cribriform nests. HE. (Inset) The neoplastic cells are positive for thyroglobulin.

Fig. 4. Anastomosing collagen bundles connecting to capsule of the tumor (Cap) are developed. The cartilages (C) are continuously formed from the fibrous tissue. HE.

Fig. 5. Multifocal cartilages (C) and bones (B) are formed in the tumor stroma. HE.

Fig. 6. The spindle cells interlacing with collagen bundles are present in the stromal fibrous area. These cells don't show atypia and mitotic figures. HE.

Fig. 7. The chondral components are composed of well-differentiated chondrocyte in mature cartilage matrices which are stained with pale to deep blue. Alcian blue staining.

Fig. 8. The osseous components are composed of mature bone matrix with osteocytes which are partly fringed with osteoblasts (arrows) and osteoclasts (arrowhead). HE.

neoplastic, but hyperplastic and metaplastic alteration. The fibroblasts, chondrocytes and osteoblasts never show atypia and mitosis and accompanying mature cartilage and bone. These components showed heterogeneous and multifocal extension in the tumor stroma without invasion to the surrounding tissues. There was no independent proliferation of the components.

Careful microscopic examination of cellular atypia, localization and invasion in the mesenchymal components to diagnose thyroid carcinoma with abundant stroma in dogs is important. In abundant stroma, it is likely that the proliferative and metaplastic mesenchymal components seem to be sarcomatous in some cases. Prognosis and therapeutic responses might be different between thyroid carcinoma and carcinosarcoma, although clinical information of carcinosarcoma is still poor because the tumor is very rare in dogs.

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