



NOTE

Pathology

Pulmonary neuroendocrine tumor in a female wolf (*Canis lupus lupus*)

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J. Vet. Med. Sci.

79(3): 588–592, 2017

doi: 10.1292/jvms.16-0490

Received: 20 September 2016

Accepted: 27 January 2017

Published online in J-STAGE:
11 February 2017

ABSTRACT. A 17-year-old female wolf (*Canis lupus lupus*) had a right lung mass that was adhered to the thoracic cavity. Histopathological examination revealed that the mass consisted of sheets, cord or ribbon-like structures of monotonous, small, cuboidal cells with round, oval or short-spindle nuclei and scant clear cytoplasm, demarcated by a fine fibrovascular stroma. Focal necrosis, congestion and thrombi were observed. Immunohistochemically, the tumor cells diffusely expressed cytokeratin AE1/AE3, and some expressed chromogranin A, neural cell adhesion molecule (CD56) and thyroid transcription factor-1. The number of proliferating cell nuclear antigen-positive tumor cells was low. A diagnosis of pulmonary neuroendocrine tumor was based on the resemblance to carcinoids.

KEY WORDS: chromogranin A, lung, NCAM (CD56), neuroendocrine tumor, wolf

Pulmonary neuroendocrine tumors are considered to be derived primarily from pulmonary neuroendocrine cells, known classically as bronchial Kulchitsky-type cells [12]. In humans, these tumors are associated with a wide prognostic spectrum, ranging from low- and intermediate-grade carcinoid tumors (typical carcinoma [TC] and atypical carcinoma [AC], respectively) to high-grade, large-cell neuroendocrine carcinomas (LCNECs) and small-cell carcinomas (SCLCs) [12]. The incidence of neuroendocrine lung tumors is extremely low in animals, including dogs [3–5] and cats [11]. We reported the case of a 17-year-old female wolf (*Canis lupus lupus*) that had pathological features of pulmonary neuroendocrine tumor that appeared to be both TC and AC.

The female wolf (*Canis lupus lupus*) was born at a zoo in Russia in 1997 and was moved to the first zoo in Japan in 1998. She was moved in 2006 to a second zoo, Hirakawa Zoological Park, where she lived for the rest of her life. She received good nutrition, and before her death, she showed no clinical signs, including respiratory abnormalities. In 2014, she was found dead, with foamy vomit on the floor.

A necropsy revealed that the anterior and middle lobes of the right lung were occupied with a large, solid, irregular-shaped mass. On cut sections, the mass had pale pink or white laminar structures, approximately 20 cm in diameter that had adhered to the thoracic wall (Fig. 1). The posterior lobe of the right lung was collapsed. The right lung mass was fixed in 10% neutral-buffered formalin, routinely processed and embedded in paraffin. Paraffin sections (5 µm) were stained with hematoxylin and eosin (H&E). The sections were also stained with Masson trichrome and Grimelius. For immunohistochemistry, sections were incubated with the following primary antibodies against cytokeratin AE1/AE3 (1:50; Dako, Glostrup, Denmark), chromogranin A (CGA, 1:3,000; Yanaihara Institute Inc., Fujinomiya, Japan), neuron-specific enolase (NSE, 1:200; Calbiochem, San Diego, CA, U.S.A.), synaptophysin (SYP, 1:500; Chemicon International Inc., Temecula, CA, U.S.A.), neural cell adhesion molecule (NCAM, CD56, 1:200; Sigma-Aldrich, St. Louis, MO, U.S.A.), thyroid transcription factor-1 (TTF-1, 1:200; Dako), proliferating cell nuclear antigen (PCNA, 1:200; Dako) and factor VIII-related antigen (1:1,000; Dako). For antigen retrieval, deparaffinized sections were heated in 10 mM citrate buffer (pH 6.0) by autoclaving at 121°C for 10 min for the SYP and TTF-1, in Dako target retrieval solution (pH 9.0, Dako Denmark A/S) by autoclaving at 121°C for 10 min for NCAM or in 10 mM citrate buffer (pH 6.0) by microwaving at 90°C for 10 min for cytokeratin AE1/AE3. Expression was detected using a VECTASTAIN® Elite ABC kit (Vector Laboratories, Inc., Burlingame, CA, U.S.A.) with 3,3'-diaminobenzidine/hydrogen peroxide as the chromogen. The sections were then counterstained with hematoxylin. For negative controls, the primary antibodies were replaced with non-immunized sera, and for positive controls, these were applied to the lung, adrenal and small intestine tissues.

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Fig. 1. Macroscopic findings of the lung tumor. The solid, irregular-shaped tumor mass with pale pink or white laminar structures spread over the thoracic cavity. Heart and lung lobes are observed close to the tumor mass in the upper left side.

Histopathological examination revealed that the mass was not encapsulated, but demarcated by a fine to thick fibrovascular stroma (Fig. 2A), which was positive for Masson trichrome. Focal necrosis and congestion were common, sometimes together with the formation of thrombi. The tumor cells were arranged in sheets (Fig. 2B), cord (Fig. 2C) or ribbon-like (Fig. 2D) structures of monotonous small columnar tumor cells, relatively small but a little larger than that of a resting lymphocyte. The tumor cells had round, oval or short-spindle nuclei with finely granular chromatin and inconspicuous nucleoli. A ribbon-like growth pattern of the tumor cells was the most common arrangement. Nuclear palisading and moulding were common; however, no mitotic figures were found in our observation. Pale eosinophilic or clear cytoplasm was very scant and showed little positive reaction for Grimelius stain, despite adequate controls. Pseudofollicular formation of tumor cells was observed; however, no typical tubular or acinar growth pattern of the tumor cells was observed in any part of the tumor mass. Immunohistochemically, the strong expression of cytokeratin AE1/AE3 was observed diffusely in the tumor mass (Fig. 3A). A patchy or focal strong expression of NSE was identified (Fig. 3B), and the weak expression was noted in the periphery of the strongly reactive areas. A similar patchy strong expression of CGA was scattered (Fig. 3C); however, the areas with CGA expression were limited, compared with the NSE expression. Nuclear expression of TTF-1 was seen in a small population of tumor cells. Membranous expression of NCAM was also observed in a very small population of tumor cells (Fig. 3D). No expression of synaptophysin and vimentin was confirmed. The number of PCNA-positive tumor cells was very low: 1.3% in 10-high-power field ($\times 400$); this was consistent with the absence of mitotic figures. Small to large vasculatures labeled with factor VIII-related antigen were observed in the interstitium of the tumor mass. Reliable immunoreactions of all the markers were confirmed in internal control tissues.

The present case was an extremely large intrathoracic mass and was characterized by histopathologic features, such as proliferation of relatively small, monotonous, round cells that were arranged primarily in a ribbon-like growth pattern. There was evidence of a pseudofollicular formation of tumor cells; however, these structures appeared to be simply derived from disassociation between tumor cells with sheet or cord-like structures. The microscopic features suggested the tumor was a neuroendocrine tumor; however, evidence of immunohistochemical neuroendocrine differentiation was limited. Non-small cell lung carcinoma, especially adenocarcinoma with neuroendocrine differentiation, has been identified in humans; a population of the tumors reacted positively with neuroendocrine markers, such as chromogranin A or synaptophysin [1, 10]. The tumor is considered a transitional form of neuroendocrine carcinoma, characterized by increasing neuroendocrine differentiation that parallels the worsening of the prognosis compared with ordinary non-small cell lung carcinoma [10]. This category might not be suitable to the present case, because our case was composed of tumor cells with only neuroendocrine morphology, but lacking morphological features of bronchiolar/alveolar adenocarcinoma. To further characterize the tumor cells, we compared the present case with dogs and other species with pulmonary neuroendocrine/carcinoid tumors. In many cases of tumors in dogs, neuroendocrine tumor cells with relatively abundant cytoplasm are arranged in an endocrine (organoid nesting) growth pattern, such as packets, lobules or sheets separated by a fibrovascular stroma [3–6, 8]. As in our case, strands or ribbon-like arrangements of the tumor cells were reported less often in the lung of a cat [11], but were observed in other organs, such as cutaneous [2] and hepatobiliary systems of cats [8, 9]. In humans, carcinoid tumors are characterized by cells with uniform cytological features and several growth patterns (i.e. organoid, trabecular, insular, palisading, ribbon and rosette-like arrangements) and highly vascularized fibrovascular stroma that suggest neuroendocrine differentiation. Such tumors are subdivided into low-grade TC and intermediate-grade AC based on the appearance of mitosis and necrosis [12]. The present case had common tumor features that are shown by both types of carcinoids;

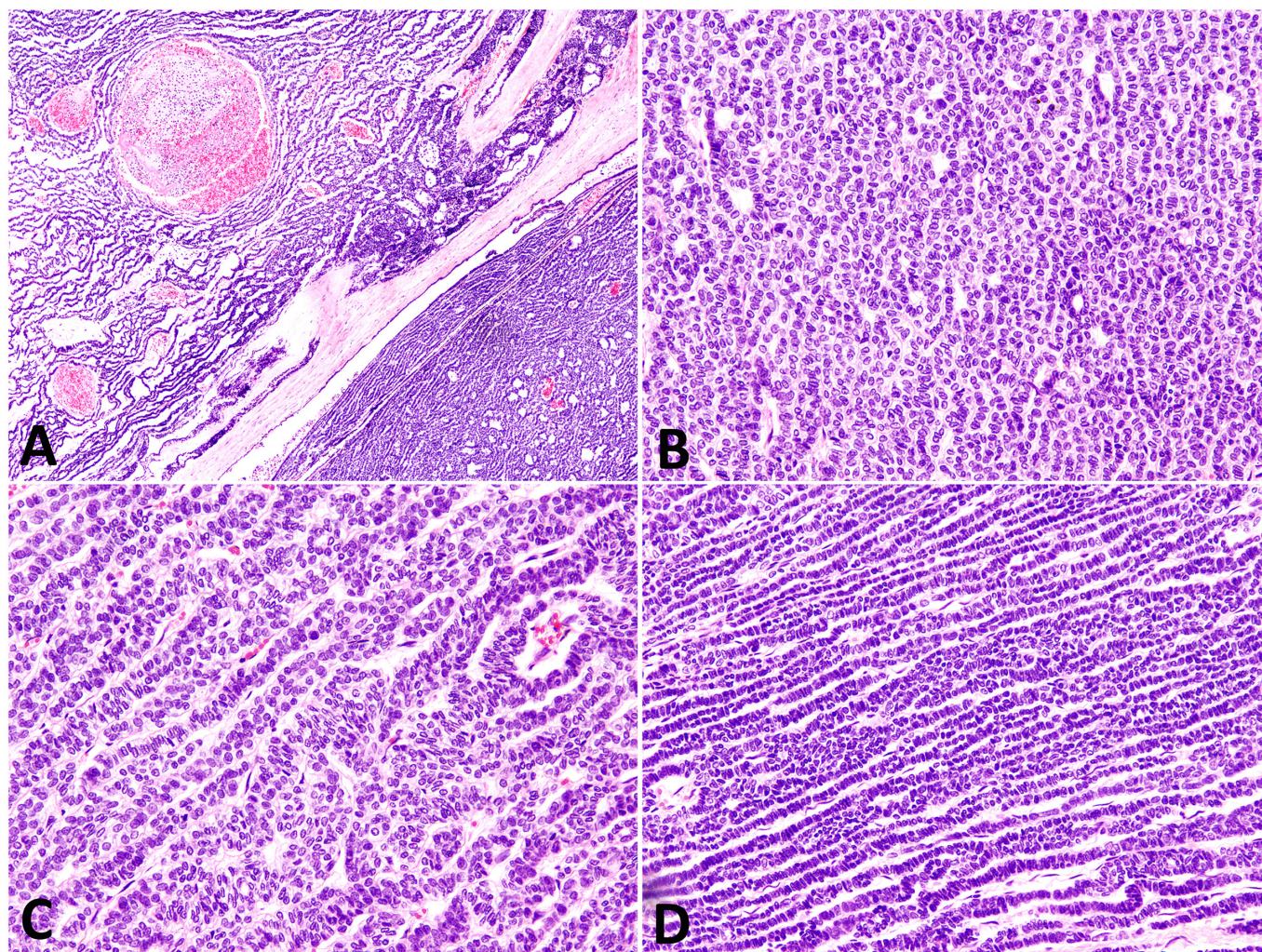


Fig. 2. Histopathological findings in the lung tumor. (A) The tumor was demarcated by thick fibrous stroma. Thrombi and congestion are seen at the upper left site. (B–D) Small monotonous tumor cells with ovoid nuclei and scant cytoplasm are arranged in a sheet (B) and cord (C) or ribbon-like (D) growth pattern. H&E stain. Bar=12.5 or 50 μ m (B–D).

key features were the presence of focal necrosis, such as AC, and a low proliferative activity of tumor cells, such as TC. Similar cases involving apparent TC [3] and AC [5] are reported in dogs. Therefore, in this case, the diagnosis made was a neuroendocrine tumor with intermediate characteristics of TC and AC, as reported in dogs.

Use of immunohistochemistry for CGA, synaptophysin, NSE and/or NCAM may help to diagnose neuroendocrine tumors [12]. The present case had a relatively limited positive reaction to CGA and NCAM and a negative reaction to synaptophysin. The data, however, were supported by previous findings of variable immunoreaction in each case of neuroendocrine tumors. For instance, a positive reaction to both CGA and synaptophysin was shown in neuroendocrine tumors that occurred in the mediastinum [7] and the carina [4] of dogs, and only a positive reaction to CGA, not synaptophysin, was shown in a lung carcinoid case in a dog [3]. NCAM is known as one of the most reliable markers for pulmonary neuroendocrine tumors in humans [12]; however, to the best of our knowledge, no such data were reported in animal tumors. Of note, the staining with TTF-1 was confirmed to be restricted in a small population of tumor cells in a similar manner to that observed in human neuroendocrine tumors [12]. Furthermore, the unique nature of these tumor cells is demonstrated by immunohistochemical responses for cytokeratin and vimentin: one case was positive for cytokeratin and vimentin [6], and another was negative for both [3]. In contrast to these findings, our case was positive for cytokeratin and negative for vimentin. These data suggest that the tumors might have a broad spectrum of cellular differentiation or are derived from different cell lineages. Even though a limited population of positive cells reacted to these selected neuroendocrine markers, the morphological features in our case facilitate the diagnosis of neuroendocrine tumor.

ACKNOWLEDGMENT. The authors are deeply grateful to Mrs. Shigeko Suzuki for her excellent technical assistance in preparing the histological specimen.

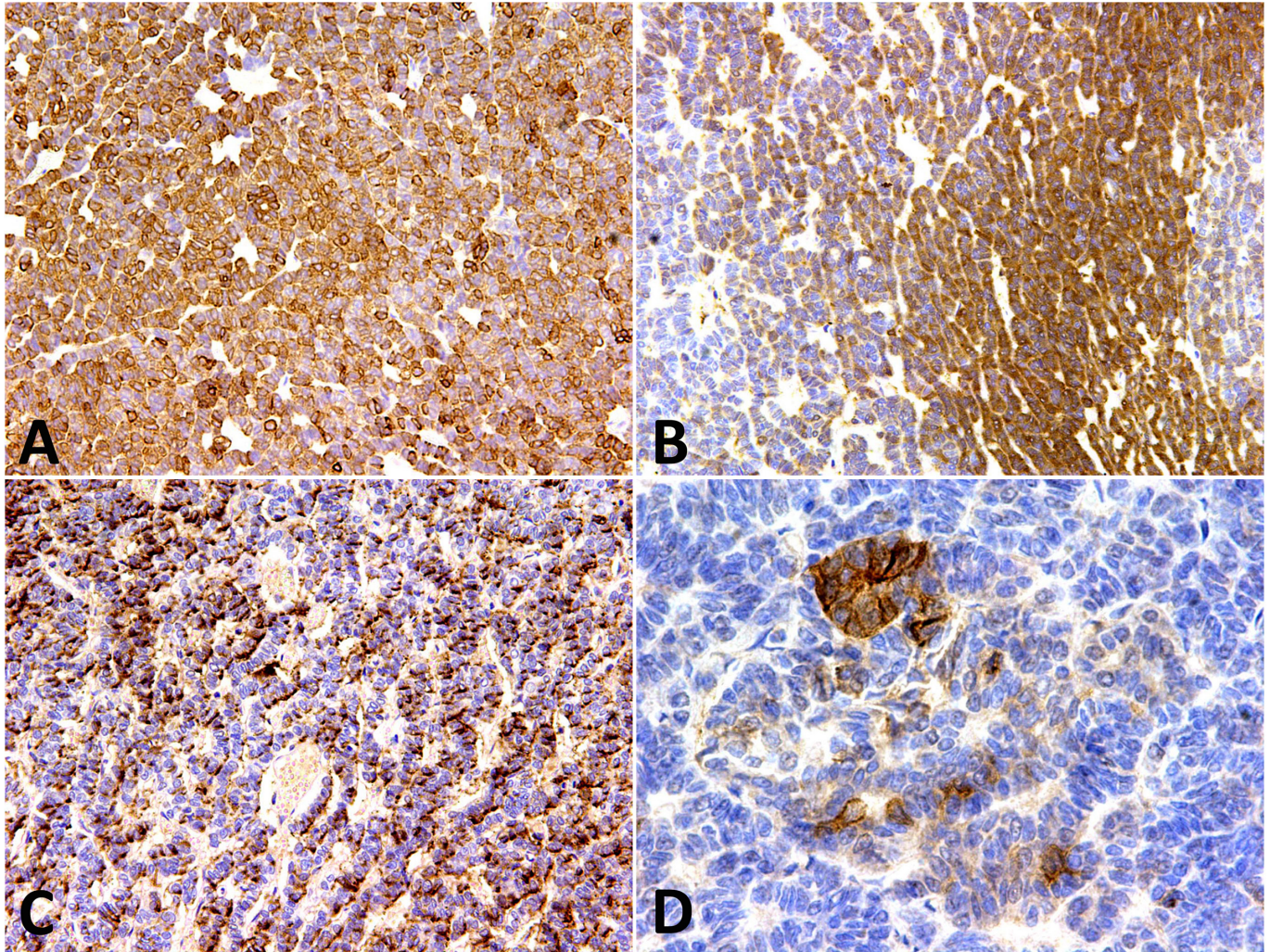


Fig. 3. Immunohistochemistry of cytokeratin AE1/AE3, NSE, CGA and NCAM in the lung tumor. (A) Strong expression of cytokeratin AE1/AE3 is observed in tumor cells. (B, C) Strong to moderate expression of NSE (B) and CGA (C) is observed in some populations of the tumor cells. (D) Strong to weak expression of NCAM (CD56) is observed in the membranes of the restricted numbers of tumor cells. Bar=50 (A–C) or 100 μ m (D).

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