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Case Report

A Neuroendocrine Carcinoma of Undetermined Origin in a Dog

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Abstract: In this report, we describe a case of neuroendocrine carcinoma of undetermined origin in a dog. Necropsy revealed scattered small neoplastic nodules in the bilateral lungs and a small nodule in the parapancreatic lymph node. Histopathologically, both pulmonary and lymph nodal nodules showed a similar histologic pattern, with neoplastic cells being arranged in diffusely proliferating sheet-like cellular nests separated by variable amounts of fibrous septa, sometimes forming rosettes and duct-like structures. Scattered small necrotic foci and invasion to fibrous septa were typically observed. Neoplastic cells showed round to oval-shaped nuclei with prominent nucleoli and abundant eosinophilic cytoplasm that were positive for Grimelius' silver impregnation staining and immunostaining with cytokeratin, synaptophysin, vasoactive intestinal peptide and chromogranin A, indicative of the development of a neuroendocrine carcinoma. However, judging from the distribution of tumors lacking the portion suggestive of the primary site in any organ examined, as well as no further indication of differentiation potential of neoplastic cells, this tumor has so far been diagnosed as neuroendocrine carcinoma of undetermined origin. (J Toxicol Pathol 2010; **23**: 151–155)

Key words: dog, neuroendocrine carcinoma, undetermined origin

Introduction

Neuroendocrine (NE) cells are derived embryologically from the gut and are widely distributed in various tissues, such as the tracheobronchial tree, liver, pancreas and genitourinary system. In humans, NE tumors (so-called carcinoids) have been found in a wide range of organs, the gastrointestinal and pulmonary tracts being the most common sites[1–3]. In domestic animals, NE tumors have occasionally been reported in the intestine, liver, bile duct, lungs, gall bladder, esophagus, skin and nasal cavity[4–13]. NE tumors usually have histopathological features forming sheets, nests or cords of small to medium-sized cells separated by delicate fibrovascular stroma to give an endocrine-type pocketing. More extensive destruction and invasion are rarely found[3,13]. Both immunohistochemical examination with antibody specific for neuropeptides and electron microscopical examination are useful for the differential diagnosis of these neoplasms in human patients and animals[14].

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This report describes the histopathological and immunohistochemical characteristics of a metastatic NE carcinoma of undetermined origin in a 12-year-old castrated male Irish setter. This dog developed generalized dermatitis accompanying pruritus, redness, alopecia and bloating of the skin, palpebra and ear canal and had received steroid chemotherapy for 11 years since one year of age. At 11 years of age, urinary bladder stones were found and surgically-removed. One year later, bladder stones were again found and surgically removed. During administration of post-surgical medication, the dog suffered sudden cardiac arrest and then died.

Necropsy revealed scattered white or grayish-white nodules measuring from 5 to 10 mm in diameter in the lung and a 2.5 cm-sized nodule occupying the parapancreatic lymph node. No other tumors/nodules were found. In addition, the mucous membrane of the urinary bladder showed scattered petechia in the pale surface. Intimal calcification of the aortic arch was also observed.

Tissue samples from tumors and all organs except for the brain, pituitary, and nasal cavity were fixed in 10% neutrally-buffered formalin (pH 7.4), routinely processed for paraffin embedding, sectioned at 4 μm and stained with hematoxylin and eosin (HE). Tissue samples from tumors were also stained by the Grimelius' silver impregnation method using a dog adrenal medulla as a positive control.

Histopathologically, neoplastic nodules in the lungs and parapancreatic lymph node showed a similar histological pattern, with neoplastic cells being arranged in diffusely proliferating sheet-like cellular nests (Fig. 1A). Small necrotic foci were scattered throughout (Fig. 1B). Neoplastic cells were separated by variable amounts of fibrous stroma, but they were invasive to the stroma and lymphatic vessels (Fig. 1C). The neoplastic cells were largely polygonal with round to oval-shaped nuclei and abundant eosinophilic cytoplasm and prominent nucleoli with indistinct cellular borders sometimes forming rosettes and duct-like structures (Fig. 1D). They were positive for Grimelius' staining with a weak to moderate distribution of tiny argyrophilic granules within the cytoplasm (Fig. 1E). Mitotic figures were observed frequently (Fig. 1D). In the lungs, a scattered distribution of neoplastic cell aggregation of various sizes was evident, as well as larger discrete nodules. In the parapancreatic lymph node, the lymphoid tissue was mostly replaced by neoplastic cells forming a large tumor mass, without invasion to the pancreatic parenchyma.

In the pancreas, scattered foci/nodules of acinar cell hyperplasia and acinar adenomas were observed. There were no other microscopically apparent neoplastic lesions in the organs/tissues examined in this case.

Sections from neoplastic tissues of the lungs and the parapancreatic lymph node were immunohistochemically stained by the avidin-biotin-peroxidase complex (ABC) procedure (Vectastain Elite ABC Kit; Vector Laboratories, Burlingame, CA, U.S.A.) and examined microscopically. Details of the specific primary antibodies used and the staining results for the neoplastic tissues are summarized in Table 1. Deparaffinized sections were blocked for endogenous peroxidase in 0.3% H_2O_2 with methanol for 30 min. Incubation of sections with the primary antibody was performed at 4°C for 16 h, followed by incubation with the biotinylated secondary antibody for 30 min, and with avidin peroxidase conjugate for 30 min at room temperature. Sections were developed in 0.05% 3,3'-diaminobenzidine/H_2O_2 solution. As positive control for each immunoreactivity, jejunum, pancreas and brain tissues from a normal dog were used. Jejunal tissue was used for confirmation of the immunoreactivity for cytokeratin (CK), synaptophysin (SYN), vasoactive intestinal peptide (VIP), chromogranin A (CGA), neuron-specific enolase (NSE), desmin, vimentin and serotonin. Pancreas was used for confirmation of glucagon, insulin and somatostatin, and brain tissue was used for S100 protein, glial fibrillary acid protein (GFAP), nestin and neurofilament (NF)-68 kDa.

Most of the neoplastic cells in tumors were diffusely immunoreactive for CGA (Fig. 1F), VIP (Fig. 1G), SYN (Fig. 1H) and CK (Fig. 1I). All of these positive immunoreactivities were observed in the cytoplasm; among them, SYN and CK showed intense immunoreactivity. On the other hand, neoplastic cells were exclusively negative for NSE, GFAP, nestin, NF-68 kDa, S100 protein, vimentin, desmin, somatostatin, glucagon, insulin and serotonin.

Immunohistochemical estimation of neuropeptides is useful in confirming neoplasms of NE-cell origin in human patients and animals[12, 13, 15]. CK, SYN, NSE and CGA are usually expressed in neoplastic cells of NE cell tumors (carcinoids) that develop in the nasal cavity, lung or gastrointestinal tract[13, 16, 17]. In addition, such neoplastic cells sometimes express serotonin, substance P, VIP, neuropeptide Y and protein gene product 9.5[18, 19]. Therefore, the positive immunoreactivity for CK, SYN, VIP and CGA in the present case strongly suggested that the NE cell was the origin of this tumor. Argyrophilic fine granular staining by the Grimelius' silver impregnation method also supported this suggestion. Moreover, negative immunoreactivity for neuronal cytoskeletal proteins, such as NF-68 kDa, class III β-tubulin and microtubule-associated protein-2, is often a feature of NE carcinomas[3, 20–22]. Also, in both human and animal cases, pulmonary, gastrointestinal and other carcinoids usually lack expression of S100 protein[23]. These observations do not conflict with our negative findings of immunoreactivity for NF and S100 in the present case.

The tumor in the present case formed scattered nodular lesions in the bilateral lungs without accompanying focal cicatricial contraction which is often considered to be the primary site of cancer development. Histopathologically, we only observed neoplastic cell infiltration into local lymphatics without formation of prominent lymphangiosis carcinomatosa, which is the typical pattern of tumor cell growth suggestive of advanced stage of lymphogenous metastases in the lung. Although we did not observe tumor emboli in the branches of the pulmonary artery, scattered multiple solitary nodules in the lungs were suggestive of hematogenous metastases from a distant origin. With regard to parapancreatic lymph node metastasis, we did not observe any visceral tumor in the abdominal cavity except for acinar cell hyperplasias and adenomas of the exocrine pancreas. These results may suggest that there is no proper candidate primary site in either the thoracic or abdominal cavities. While there were no accompanying clinical signs, the nasal mucosa and pituitary, which we did not examine at necropsy, could be candidates for the primary site. These unexamined organs/tissues contain neuroendocrine cells, and NE carcinomas with metastases have also been reported in dogs or humans[24, 25].

In summary, we report here a case of malignant tumor of undetermined origin in a dog. Because of typical histological and immunohistochemical features of neoplastic cells, this tumor was diagnosed as NE carcinoma. Because of the scattered distribution pattern of neoplastic nodules, the involvement of the lungs and parapancreatic lymph node is considered to be metastatic; however, there was no candidate visceral organ/tissue for the primary site of this tumor in the thoracic or abdominal cavities. Although there were no accompanying clinical signs, the cranial or nasal cavity could be considered as the origin in the present case.

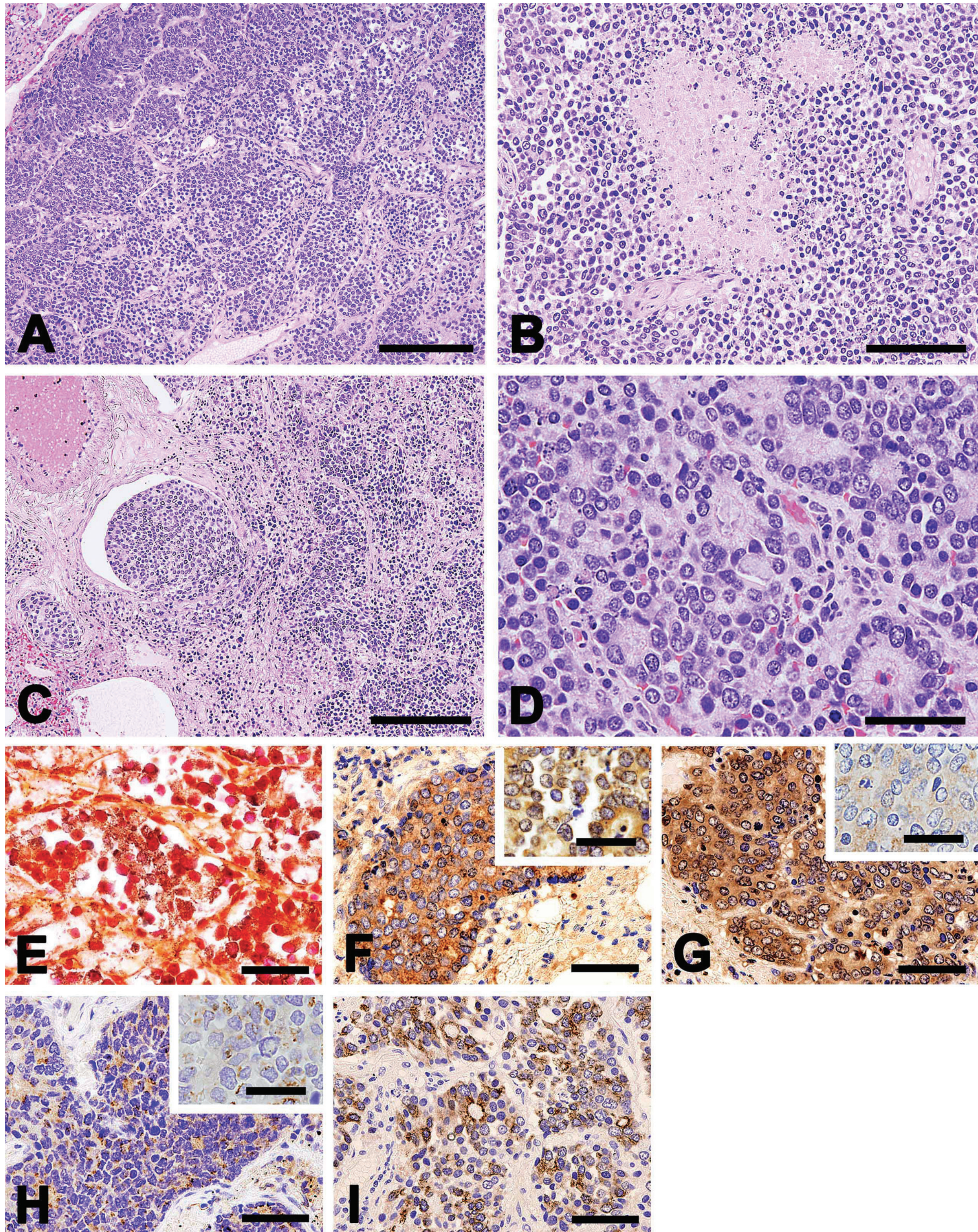

Fig. 1. Histological and immunohistological features of pulmonary neoplastic nodules. Neoplastic cells were arranged in a diffusely proliferating sheet-like cellular nest separated by various amounts of fibrous septa (A) associated with scattered necrotic foci (B). A: Bar=200 μm. B: Bar=100 μm. C: Neoplastic cells were separated by variable amounts of fibrous stroma, but they were invasive to the stroma and lymphatic vessels. Bar=200 μm. D: Neoplastic cells were largely polygonal with round to oval-shaped nuclei, abundant eosinophilic cytoplasm and prominent nucleoli with indistinct cellular borders, sometimes forming rosettes and duct-like structures. Mitotic figures were observed frequently. Bar=50 μm. E: Neoplastic cells were positive for Grimelius' staining with a weak to moderate distribution of tiny argyrophilic granules within the cytoplasm. Bar=50 μm. F–H: Neoplastic cells showed less clear granular cytoplasmic immunoreactivity with CGA (F), VIP (G) and SYN (H). Bar=50 μm (inset =30 μm), Bar of middle power field=30 μm. I: Neoplastic cells also showed strong cytoplasmic immunoreactivity with CK. Bar=50 μm.

Table 1. Results of Immunohistochemical Staining of the Tumor in the Irish Setter

Antigen	Host species	Clonality [clone]	Dilution	Antigen retrieval	Supplier	Reactivity[a]
Cytokeratin[b] (CK)	Mouse	Mono [MNF116]	1: 200	MW[c]	Dako, Glostrup, Denmark	+++
Synaptophysin (SYN)	Mouse	Mono [SY38]	1: 1,000	AC[d]	Chemicon International, Temecula, CA, U.S.A.	+++
Vasoactive intestinal peptide (VIP)	Rabbit	Poly	1: 500	MW[c]	Chemicon International	++
Chromogranin A (CGA)	Rabbit	Poly	1: 300	MW[c]	Yanaihara Institute, Shizuoka, Japan	++
Neuron-specific enolase (NSE)	Rabbit	Poly	1: 300	None	Calbiochem, San Diego, CA, U.S.A.	–
S100 protein	Rabbit	Poly	1: 100	None	Thermo Fisher Scientific, Fremont, CA, U.S.A.	–
Glial fibrillary acid protein (GFAP)	Mouse	Mono [G-A-5]	1: 200	None	Chemicon International	–
Nestin	Mouse	Mono [2Q178]	1: 100	MW[c]	Abcam, Cambridge, MA, U.S.A.	–
Neurofilament 68 kDa (NF-68KDa)	Mouse	Mono [NR4]	1: 400	MW[c]	Sigma, St. Luis, MO, U.S.A.	–
Desmin	Mouse	Mono [D33]	1: 50	MW[c]	Affinity BioRegents, Rockford, IL, U.S.A.	–
Vimentin	Rabbit	Poly	1: 200	MW[c]	Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.	–
Glucagon	Rabbit	Poly	1: 400	None	Dako	–
Insulin	Guinea Pig	Poly	1: 300	MW[c]	Nichirei Biosciences Inc., Tokyo, Japan	–
Serotonin	Rabbit	Poly	prediluted	None	Dako	–
Somatostatin	Rabbit	Poly	1: 500	None	Dako	–

[a] –, negative; +, scattered or sparse; ++, moderate but diffuse; +++, strong but diffuse.
[b] This antibody recognized cytokeratin 5, 6, 8, 17 and 19.
[c] Antigen retrievals were performed in a microwave at 90°C in 10 mM citrate buffer (pH 6.0) for 10 min.
[d] Antigen retrievals were performed in a autoclave at 121°C in 10 mM citrate buffer (pH 6.0) for 10 min.

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