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SPONTANEOUSLY ARISING DISEASE

Diffuse Pulmonary Meningotheliomatosis with Sarcomatous Transformation in a Shiba Dog

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Summary

A 2-year-old neutered female Shiba dog exhibited laboured breathing for 1 month. Computed tomography of the thoracic cavity revealed multiple nodules (2–5 mm diameter) in the lungs. Grossly, the lungs were firm and normal in shape. The nodules were grey–white in colour. Microscopically, the nodules were non-encapsulated and exhibited an irregular shape. They were composed of polygonal or spindle cells with indistinct cell borders arranged in sheets. The cells had large, round, hyperchromatic nuclei and abundant pale eosinophilic cytoplasm with no atypia. Intrapulmonary arterial emboli and infiltration into the bronchioles were observed. Immunohistochemically, the cells were positive for vimentin and negative for cytokeratin, glial fibrillary acidic protein and α-smooth muscle actin. Ultrastructurally, the cells displayed cytoplasmic processes, desmosomes and intermediate filaments. These findings led to a diagnosis of diffuse pulmonary meningotheliomatosis with sarcomatous transformation. To the best of our knowledge, this is the first report of diffuse pulmonary meningotheliomatosis in a dog.

Keywords: diffuse pulmonary meningotheliomatosis; dog; lung; sarcomatous transformation

Minute pulmonary meningothelial-like nodules (MPMNs) are detected incidentally in resected lungs or on autopsy of human patients (Korn *et al.*, 1960; Mizutani *et al.*, 2009). MPMNs were identified in 7.0% (121/1,724 cases) of patients undergoing lung resection and were more common in elderly women than in men (Mizutani *et al.*, 2009). MPMNs, initially described as pulmonary tumours resembling chemodectomas, have been recognized as a tumour-like disease consisting of epithelioid nests or whorls in a Zellballen-like arrangement centred on small veins (Korn *et al.*, 1960; Ionescu *et al.*, 2004). Diffuse pulmonary meningotheliomatosis (DPM), which diffusely presents as multiple MPMNs in both lungs, is a rare pulmonary disease in man (Suster and Moran, 2007; Mora *et al.*, 2013; Huang *et al.*, 2015). DPM is difficult to distinguish from infectious diseases, such as tuberculosis and metastatic tumours, presenting as diffuse millet-sized nodules in the lungs on computed tomography (CT). MPMNs and DPM have not been described in domestic animals to date. To the best of our knowledge, this is the first report of diffuse pulmonary meningotheliomatosis in a dog.

A 2-year-old neutered female Shiba dog displayed laboured breathing for 1 month. Haematological

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and serum biochemical parameters were within normal limits. Bacteriological and fungal cultures of thoracentesis fluid were negative. Radiography and CT of the thoracic cavity revealed multiple nodules (2–5 mm diameter) in both lungs and normal pulmonary lymph nodes (Fig. 1). A part of the left cranial lung lobe was removed surgically for histopathological and microbiological examination. Twelve pathogenic causes of respiratory disorders, including *Bordetella bronchiseptica*, canine distemper virus, canine herpesvirus, canine respiratory coronavirus and *Mycoplasma cynos*, were not detected using a polymerase chain reaction respiratory disease panel (IDEXX Laboratory, Tokyo, Japan). Twenty-seven hours after the pulmonary excision, the dog was humanely destroyed. Although the left cranial lung lobe was collected immediately, necropsy examination could not be performed.

Grossly, the lungs were irregular in shape and firm in consistency. Multiple nodules (2–5 mm diameter) with grey–white surface were observed (Fig. 2).

Pulmonary tissue was fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections (4 μm) were stained with haematoxylin and eosin (HE), periodic acid–Schiff (PAS), Masson's trichrome, Ziehl–Neelsen (ZN) and Grimelius stains. Serial sections were subjected to immunohistochemistry (IHC) using the labelled streptavidin–biotin method and the primary antibodies listed in Supplementary Table 1. The sections were treated with 0.03% H_2O_2 in 33% methanol at room temperature for 30 min to block endogenous peroxidase, followed by antigen retrieval. Labelling was 'visualized' by adding 3, 3′-diaminobenzidine tetrahydrochloride as chromogen and counterstaining with haematoxylin. The primary antibodies were validated through positive reactions with appropriate normal tissue control sections and negative reactions when normal mouse or rabbit immunoglobulin G was substituted for antibody. For electron microscopical examination, small pieces of formalin-fixed pulmonary tissue were re-fixed in 1% osmium tetroxide followed by 0.2 M phosphate buffer and then embedded in epoxy resin. Using an electron microscope (JEM-1011; JEOL, Tokyo, Japan), ultrathin sections were examined after staining with uranyl acetate and lead citrate.

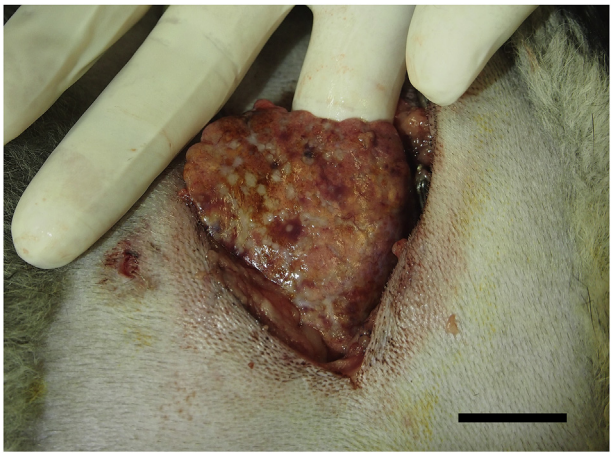

Fig. 2. The left cranial lung lobe before collection. The lungs had an irregular shape. Multiple nodules (5 mm diameter) with grey–white surface are observed. Bar, 3 cm.

Microscopically, the nodules were non-encapsulated and had an irregular shape. They were composed of polygonal or spindle cells with indistinct cell borders arranged in sheets (Supplementary Fig. 1). The cells had large, round hyperchromatic nuclei and abundant pale eosinophilic cytoplasm with no atypia (Fig. 3). The cells

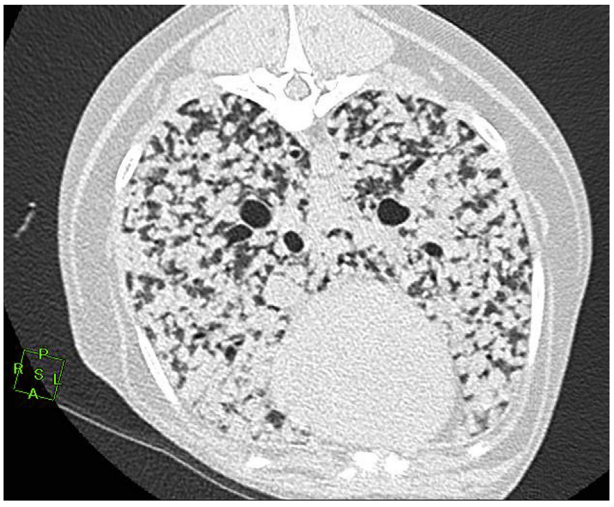

Fig. 1. Computed tomography of the thoracic cavity reveals multiple nodules measuring 2–5 mm in diameter in both lungs.

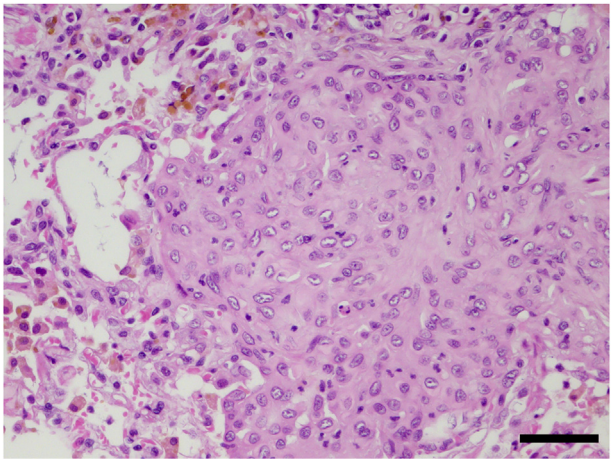

Fig. 3. The nodules consist of polygonal cells with indistinct cell borders arranged in a sheet. HE. Bar, 50 μm.

formed intrapulmonary arterial emboli and infiltrated into the bronchioles (Fig. 4). The cells were negative for PAS, ZN and Grimelius stains. Clear pseudonuclear inclusions and mitotic figures were not observed. Inflammatory cells, such as foamy or haemosiderin-laden macrophages and neutrophils and small foci of necrosis were often found in the nodules. The alveoli surrounding the nodules also contained a few haemosiderin-laden macrophages. The histology of the pulmonary tissue obtained by biopsy was similar to that of tissue obtained after humane destruction. Immunohistochemically, the cells were positive for vimentin (Supplementary Fig. 2) and negative for cytokeratin (CK) AE1/AE3 (Supplementary Fig. 3), progesterone receptor (PR), chromogranin A, CD204, S100, glial fibrillary acidic protein, synaptophysin, neuron-specific enolase, ionized calcium binding adaptor molecule-1, thyroid transcription factor-1, α-smooth muscle actin, E-cadherin and factor VIII-related antigen. The alveolar epithelial cells surrounding the nodules were positive for CK AE1/AE3 (Supplementary Fig. 3). The Ki67 labelling index of the cells comprising the nodules was approximately 2%. Ultrastructurally, cytoplasmic processes, desmosomes and intermediate filaments were observed (Supplementary Fig. 4). No dense-core granules or other distinctive organelles were identified.

On the basis of the gross, microscopical, immunohistochemical and ultrastructural findings, the lesion was diagnosed as diffuse pulmonary meningotheliomatosis with sarcomatous transformation. The proliferating cells observed in the present case resembled those in MPMN and DPM in man, including the expression of vimentin and the presence of intracytoplasmic processes and desmosomes (Gaffey *et al.*, 1988; Pelosi *et al.*, 2002; Suster and Moran, 2007). However, the cellular origin of MPMNs and DPM remains unclear. Several studies revealed similarities with meningothelial cells on the basis of immunohistochemical and ultrastructural findings (Gaffey *et al.*, 1988; Suster and Moran, 2007). In contrast, Torikata and Mukai (1990) reported MPMNs with myosin expression, supporting a myogenic origin. In MPMNs and DPM, vimentin, PR and epithelial membrane antigen (EMA) are useful markers for immunohistochemically characterizing the proliferating cells of the nodules (Gaffey *et al.*, 1988; Suster and Moran, 2007). Unfortunately, in the present study, the commercial antibody against EMA did not cross-react with the protein in the canine pulmonary tissue. This case should be distinguished diagnostically from metastatic tumours, including intracranial meningioma and non-neoplastic multiple proliferative lesions, including sarcoidosis, inflammatory pseudotumour, micronodular pneumocyte hyperplasia and proliferating mesothelial cells. Intracranial meningioma with pulmonary metastasis has been reported in three dogs, which presented with clinical signs including ataxia and seizures (Schulman *et al.*, 1992). In the present case, metastatic meningioma was excluded owing to the presence of respiratory signs, the absence of neurological signs, the lack of enlarged pulmonary lymph nodes on CT, the presence of lung nodules and a low proliferative activity index, although CT imaging of the head and neck was not performed. Primary meningioma arising from the lungs as described in man has not been reported in dogs. Sarcoidosis, the lesions of which predominantly comprise of epithelioid macrophages with lymphocytes, is a systemic granulomatous disease of undetermined aetiology (Nunes *et al.*, 2005). Inflammatory pseudotumours form nodules comprised of various cell populations, including macrophages, plasma cells and fibroblasts (Matsubara *et al.*, 1998). Micronodular pneumocyte hyperplasia is a papillary proliferation of type II alveolar epithelial cells expressing CK and EMA and it is observed in the lungs of patients with nodular sclerosis (Maruyama *et al.*, 2001). Microvilli, in addition to desmosomes and cytoplasmic processes, are observed in the proliferating mesothelial cells (Hjelle *et al.*, 1989). Therefore, these differential diagnoses were excluded in the present case.

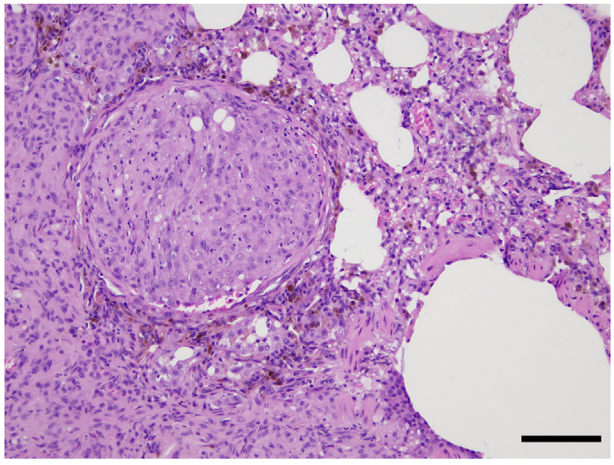

Fig. 4. Intrapulmonary arterial emboli formed of the proliferating cells constituting the nodules. HE. Bar, 100 µm.

The cells comprising MPMNs and DPM resemble meningothelial cells on the basis of their immunohistochemical and ultrastructural features (Gaffey *et al.*, 1988; Suster and Moran, 2007). MPMNs have been considered to be the result of reactive proliferation, because a polyclonal

population, in addition to a monoclonal population, was confirmed via clonality analysis based on an X-chromosome-linked polymorphic marker, the human androgen receptor gene (Niho *et al.*, 1999). Conversely, in DPM, loss of heterozygosity is frequently detected in various chromosomal loci, such as 1p, 3p, 9p, 10q, 17p and 22q, triggering inactivation of tumour suppressor genes, including *VHL*, *p16*, *p53* and *NF2*, suggesting the transition between reactive and neoplastic proliferation or the acquisition of neoplastic proliferation capacity (Ionescu *et al.*, 2004). The mean Ki67 index of MPMNs, denoting proliferative activity, is 0.38% (range, 0.3–0.5%, six cases), while that of DPM is <2% (Pelosi *et al.*, 2002; Gleason *et al.*, 2016). However, the microscopical features of neoplastic transformation, such as arterial emboli and infiltration into the bronchioles of the proliferating cells and the higher Ki67 index of meningothelial-like cells, have been not described in human DPM. In the present case, microscopical findings indicating a malignant tumour were observed, suggesting sarcomatous transformation of proliferating cells, although genetic aberration analysis was not performed in this study.

The pathogenesis of MPMNs has suggested an association with circulatory disturbance and hormones (Korn *et al.*, 1960; Zak and Chabes, 1963; Pelosi *et al.*, 2002). MPMNs are detected incidentally in resected lungs or at autopsy and are recognized with pulmonary tumours and pulmonary hypertension caused by perivascular nodules (Korn *et al.*, 1960; Zak and Chabes, 1963; Niho *et al.*, 1999; Mizutani *et al.*, 2009). However, treatment specific to MPMNs is not available. In addition, the proliferating meningothelial-like cells of MPMNs express PR, suggesting sex steroid-mediated control of the cell growth (Gaffey *et al.*, 1988; Pelosi *et al.*, 2002; Suster and Moran, 2007). The significance of the absence of PR expression in the present case remains unclear. In human DPM, there are various clinical signs such as breathlessness, although some cases can be asymptomatic, indicating that symptoms may be dependent on the size and number of nodules (Suster and Moran, 2007; Mora *et al.*, 2013). In contrast, diffuse multiple nodules arising from all parts of the lungs, as observed in the present case, have not been described in human patients. It is difficult to distinguish between inflammatory disorders, including non-neoplastic proliferative lesions and neoplastic lesions, via CT and microbiological examination. Therefore, histopathological examination may be necessary for a definitive diagnosis of MPMNs and DPM.

Conflict of Interest Statement

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcpa.2019.06.005.

References

Gaffey MJ, Mills SE, Askin FB (1988) Minute pulmonary meningothelial-like nodules. A clinicopathologic study of so-called minute pulmonary chemodectoma. *American Journal of Surgical Pathology*, **12**, 167–175.

Gleason JB, Valentin R, Almeida P, Martinez N, Bejarano PA (2016) Diffuse pulmonary meningotheliomatosis: a literature review of a rare diffuse parenchymal lung disease with unclear clinical significance. *Journal of the Association of Chest Physicians*, **5**, 18–25.

Hjelle JT, Golinska BT, Waters DC, Steidley KR, McCarroll DR *et al.* (1989) Isolation and propagation *in vitro* of peritoneal mesothelial cells. *Peritoneal Dialysis International*, **9**, 341–347.

Huang EC, Zhang Y, Bishop JW, Gandour-Edwards RF, Afify AM (2015) Diffuse pulmonary meningotheliomatosis: a diagnostically challenging entity on fine-needle aspiration cytology. *Diagnostic Cytopathology*, **43**, 727–730.

Ionescu DN, Sasatomi E, Aldeeb D, Omalu BI, Finkelstein SD *et al.* (2004) Pulmonary meningothelial-like nodules: a genotypic comparison with meningiomas. *American Journal of Surgical Pathology*, **28**, 207–214.

Korn D, Bensch K, Liebow AA, Castleman B (1960) Multiple minute pulmonary tumors resembling chemodectomas. *American Journal of Pathology*, **37**, 641–672.

Maruyama H, Seyama K, Sobajima J, Kitamura K, Sobajima T *et al.* (2001) Multifocal micronodular pneumocyte hyperplasia and lymphangioleiomyomatosis in tuberous sclerosis with a TSC2 gene. *Modern Pathology*, **14**, 609–614.

Matsubara O, Tan-Liu NS, Kenney RM, Mark EJ (1988) Inflammatory pseudotumors of the lung: progression from organizing pneumonia to fibrous histiocytoma or to plasma cell granuloma in 32 cases. *Human Pathology*, **19**, 807–814.

Mizutani E, Tsuta K, Maeshima AM, Asamura H, Matsuno Y (2009) Minute pulmonary meningothelial-like nodules: clinicopathologic analysis of 121 patients. *Human Pathology*, **40**, 678–682.

Mora RB, Nieto JMS, Hu C, Mateos EA, Bascuñana AG *et al.* (2013) Diffuse pulmonary meningotheliomatosis diagnosed by transbrochial lung biopsy. *Respiration*, **86**, 145–148.

Niho S, Yokose T, Nishiwaki Y, Mukai K (1999) Immunohistochemical and clonal analysis of minute pulmonary

meningothelial-like nodules. *Human Pathology*, **30**, 425–429.
Nunes H, Soler P, Valeyre D (2005) Pulmonary sarcoidosis. *Allergy*, **60**, 565–582.
Pelosi G, Maffini F, Decarli N, Viale G (2002) Progesterone receptor immunoreactivity in minute meningothelioid nodules of the lung. *Virchow's Archive*, **440**, 543–546.
Schulman FY, Ribas JL, Carpenter JL, Sisson AF, LeCouteur RA (1992) Intracranial meningioma with pulmonary metastasis in three dogs. *Veterinary Pathology*, **29**, 196–202.
Suster S, Moran CA (2007) Diffuse pulmonary meningotheliomatosis. *American Journal of Surgical Pathology*, **31**, 624–631.
Torikata C, Mukai M (1990) So-called minute chemodectoma of the lung. An electron microscopic and immunohistochemical study. *Virchow's Archive*, **417**, 113–118.
Zak FG, Chabes A (1963) Pulmonary chemodectomatosis. *Journal of the American Medical Association*, **183**, 887–889.

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