Pathology in Practice

In collaboration with the American College of Veterinary Pathologists

History

A 5-month-old castrated male ferret (*Mustela putorius furo*) was obtained from a commercial vendor for use in pediatric intubation teaching at a research hospital. Instructional activities and animal care were conducted under Institutional Animal Care and Use Committee regulations. On arrival, physical examination was unremarkable, except for diarrhea that tested negative for ferret enteric coronavirus by PCR assay. Over the following 4 months, the ferret's diarrhea resolved, and weight gain was clinically normal. The ferret was used in intubation laboratories twice (at 11 and 16 month old). At approximately 20 months of age, the ferret had intermittent weight loss, which resolved with supportive care (lactated Ringer's solution) and supplemental feed (ferret treats and canned cat food). At approximately 2 years old, the ferret was transferred to a veterinary technician teaching program for use in animal handling laboratories and was soon thereafter adopted to a private home. At approximately 3 years of age, the ferret was presented because of lethargy, anorexia, and weight loss.

Clinical and Gross Findings

Physical examination revealed poor body condition and pale mucous membranes. Multiple, small masses were palpated in the abdomen. A left lateral radiographic image of the ferret revealed abundant abdominal fluid. The prognosis was poor, and euthanasia was elected for humane reasons.

At necropsy, multiple variably-sized (0.2 to 1 cm diameter), firm to soft, white nodules expanded the greater and lesser omentum and the gastrosplenic

Figure 1—Postmortem photograph of the abdominal organs of a 3-year-old castrated male ferret (*Mustela putorius furo*) examined because of lethargy, anorexia, and weight loss. Diffusely, the greater omentum and the gastrosplenic mesentery are expanded by myriad, small, firm to soft, white nodules. The liver (arrowhead) and spleen (arrow) are also pictured.

mesentery (**Figure 1**). Several small, white nodules were present on the marginal splenic capsule and on the abdominal aspect of the diaphragm. The capsular surface of the kidney was irregular and pale, and wedge-shaped lesions were present on cut section. No tumors were identified in the stomach, pancreas, liver, or other organs. Samples of omentum, diaphragm, lungs, kidney, stomach, small intestine, colon, and spleen were collected, fixed in neutral-buffered 10% formalin, and processed for microscopic evaluation.

Formulate differential diagnoses, then continue reading.

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<https://doi.org/10.2460/javma.19.12.0642>

Figure 2—Postmortem photomicrographs of tissue sections of multifocal tumor associated with the small intestine (A and B) and diaphragm (C and D). A—A nodule on the visceral surface of the small intestine is in the center of the image. H&E stain; bar = 2,000 μ m. B—Most of the nodule is composed of branching fronds and papillary projections of cuboidal to columnar neoplastic cells. H&E stain; bar = 100 μ m. C—Neoplastic cells with epithelioid morphology line papillary structures (arrow), and plump, spindle-shaped cells form solid groups (arrowhead). H&E stain; bar = 20 μ m. D—In one focus in the diaphragm, groups of neoplastic cells form intravascular tumor emboli. Note the endothelial cell nucleus (arrow) that confirms this intravascular location. H&E stain; bar = 20 μ m.

Histopathologic Findings

Histologically, the omentum was expanded by multiple, coalescing nodules composed of epithelioid cells arranged in a single layer on branching papillary projections of scant fibrovascular stroma or were present in clusters and aggregates of plump spindlyoid cells (**Figure 2**). Similar groups of neoplastic cells were present on visceral serosal surfaces, invaded between and compressed skeletal muscle of the diaphragm, and, rarely, were present within vascular spaces. Epithelioid cells were cuboidal to columnar with a moderate amount of homogeneous, eosinophilic cytoplasm. Nuclei of epithelioid cells were round to ovoid to indented with coarsely clumped to vesicular chromatin and prominent, basophilic nucleoli. There was moderate variation in size and shape of nuclei (3X anisokaryosis with round to oval nuclei). Bi- and multinucleated cells were present. Among epithelioid cells, the mitotic count was 2 mitotic figures in 2.37 mm² (the area in 10 hpf [400X]). Solid areas of tumor were composed of sheets of plump, spindlyoid cells with indistinct cell borders, scant to moderate, homogeneous, eosinophilic cytoplasm, and ovoid nuclei with coarsely clumped chromatin and 1 or 2 nucleoli. Mitotic count among spindlyoid regions of the tumor was 1 mitotic figure in 2.37 mm². Areas of hemorrhage, hemosiderosis, necrosis, and fibrin accumulation were also present.

Immunohistochemical staining was used to characterize the neoplasm (**Figure 3**). Indirect immunostaining protocols were employed with antibodies directed against cytokeratin AE1/AE3 (anti-AE1/AE3 antibody, bs-1712R, Bioss Antibodies Inc),

vimentin (anti-vimentin antibody, ab22651, Abcam), Wilms' tumor-1 (WT1; anti-WT1 antibody, ab89901, Abcam), a polymer detection system (Bond Polymer Refine Detection, Leica Biosystems Inc) as secondary reagent, diaminobenzidine as chromogen, and hematoxylin stain for counterstain. Immunofluorescence staining was performed using anti-WT1 antibody detected by goat anti-rabbit IgG (Alexa Fluor 488, ab150077, Abcam) and nuclei detection (Fluoroshield mounting medium with DAPI, ab104139, Abcam). A fluorescent phalloidin conjugate (Cytopainter Phalloidin-iFluor 555, ab176756, Abcam) was used to demonstrate polymerized actin of the cytoskeleton. Kidney tissue from this patient, as well as murine, porcine, and macaque kidney tissue, served as positive controls for WT1 immunostaining. Omission of the primary antibody was used as a negative control. Both epithelioid and spindle-shaped tumor cells had moderately strong, positive cytoplasmic staining of cytokeratin and vimentin, and weak to moderate, punctate nuclear staining of WT1 in scattered neoplastic cells, consistent with mesothelioma.¹⁻³ Computer-aided image analysis using a nuclear algorithm (Leica Biosystems Inc) revealed that, in some regions of the tumor, up to 85% of tumor cell nuclei were faintly (1+) or moderately (2+) immunopositive for WT1.

Lesions in other organs included moderate lymphocytic gastroenteritis with regional mucosal dysplasia and scattered cystic dilated glands with degenerating inflammatory cells. In Giemsa-stained sections, short, spiral-shaped bacteria typical of *Helicobacter* spp were observed in gastric glands in inflamed areas of the antral region. Chronic renal

Figure 3—Photomicrographs of immunohistochemical analysis of neoplastic mesothelial cells from the ferret described in Figure 1. A—Expression of cyokeratin is shown. Immunostaining with anti-AE1/AE3 antibody (bs-1712R, Bioss Antibodies Inc) and hematoxylin counterstain; bar = 20 μ m. B—Expression of vimentin is shown. Immunostaining with anti-vimentin antibody (ab22651, Abcam) and hematoxylin counterstain; bar = 20 μ m. C and C inset—Expression of the nuclear protein, Wilms' tumor-1 (WT1), by neoplastic mesothelial cells (arrows; C) and positive control (ferret glomerular podocytes; inset) is shown. Both epithelioid and mesenchymal components of the tumor have cells that express WT1 at higher levels than the positive control glomerular podocytes. Immunostaining with anti-WT1 antibody (ab89901, Abcam), a polymer detection system as secondary reagent, diaminobenzidine as chromogen, and hematoxylin counterstain;

bar = 20 μ m. D—Nuclei (blue) of neoplastic mesothelial cells forming papillary fronds have variably intense punctate, nuclear staining (green), indicating the presence of WT1. Cytoskeleton appears red. Immunofluorescence staining for WT1 (Alexa Fluor 488, ab150077, Abcam), nuclei detection (ab104139, Abcam), and polymerized actin of the cytoskeleton (CytosPainter Phalloidin-iFluor 555, ab176756, Abcam) with hematoxylin counterstain; bar = 20 μ m.

infarcts and chronic glomerulonephritis were also present. Lesions were not observed in the lungs.

Morphologic Diagnosis and Case Summary

Peritoneal mesothelioma with diaphragmatic invasion, intravascular tumor emboli, and a biphasic microscopic pattern in a juvenile ferret. Neoplastic cells expressed cyokeratin, vimentin, and the nuclear regulatory protein WT1.

Comments

Mesotheliomas are aggressive neoplasms arising from mesodermal cells that normally form a monolayer covering the serosal surfaces of body cavities and viscera. In human medicine, mesothelioma was extremely rare until the widespread use of asbestos in industry and building construction in the late 19th century. Exposure to this silicate material sometimes results in malignant transformation of mesothelial cells, probably through the induction of inflammation and genetic defects.⁴ Although mesothelioma is classically linked to asbestos exposure in human beings and, possibly, in companion dogs,⁵ a relationship between asbestos exposure and development of mesothelioma has not been reported in other animal species.

The most frequently reported clinical signs of mesothelioma in human patients are abdominal pain, swelling, or both; anorexia; weight loss; and ascites.^{4,6} Mesothelioma has a long-latency period and is often diagnosed in the advanced stages of disease, mainly due to the nonspecific features of clinical and radiographic presentation. Diagnostic imaging can suggest the presence of mesothelioma, but histologic evaluation and, usually, immunohistochemical characterization are required for a definitive diagnosis.¹ Conventional treatments are often ineffective. Options for effective treatment or management for human patients are under intense research and include chemotherapy (permetrexed, cisplatin), adjunctive antiangiogenesis agents (bevacizumab, nintedanib), as well as agents that target immune responses, mesothelin, and other molecular pathways.^{4,7,8} Radiotherapy is used for palliative care. Currently, the usefulness of surgery in the management of mesothelioma is debatable.^{7,9-12}

To our knowledge, reports of mesothelioma in domestic ferrets are limited to 2 that originated in the peritoneum and 1 in the pleura.¹³⁻¹⁶ Top differential diagnoses for a 3-year-old ferret with weight loss and ascites include ferret coronavirus infection, intestinal foreign body, lymphoma, insulinoma, and gastric adenocarcinoma, based on the more common diseases reported in the domestic ferret.¹⁷⁻¹⁹ In this case, abdominal distension and other clinical signs can be attributed to the malignant peritoneal mesothelioma.

Mesotheliomas may arise from any structure lined or covered by mesothelium, including the thoracic, abdominal, and vaginal cavities and their viscera. In human beings, development of mesothelioma in the abdomen is less common than pleural origin, with approximately one-third arising from the peritoneum.²⁰ The location of mesothelioma in dogs seems to be fairly evenly mixed, with pleural mesotheliomas being slightly more common than abdominal mesotheliomas.²¹ In cattle, mesothelioma appears most often in the peritoneal cavity.²² The tunica vaginalis testis is a frequent site of spontaneous and experimentally induced mesotheliomas in rats.^{20,23}

In the ferret of the present report, the tumor had a mixture of neoplastic cells with epithelial and mesenchymal cytology, consistent with the biphasic subtype of mesothelioma. Immunohistochemical features of this ferret's tumor were in agreement with malignant mesotheliomas in other species. Concurrent expression of cytokeratin and vimentin intermediate filaments reflects the plasticity of mesothelial cells.²⁴ The nuclear protein WT1 is overexpressed in > 90% of malignant pleural mesotheliomas in human patients and is a regulator of epithelial-to-mesenchymal (EMT) transition and its reverse process, mesenchymal-to-epithelial (MET) transition.²⁵ This protein functions as a regulator of transcription of growth-promoting genes and is important in the development of cells derived from the embryonic mesoderm.^{26,27} The mechanisms by which WT1 may affect the development or metastasis of mesothelioma in human and animal patients are unknown. Although WT1 has long been known to behave as a classic tumor suppressor gene in nephroblastoma, it also may have a role as an oncogene²⁵ in other neoplasms, which might explain increased WT1 expression in mesothelioma. Across the Animal Kingdom, there is a very high degree of homology among amino acid sequences for WT1, with > 94% identity between ferret and human isoforms (basic local alignment search tool analysis performed on XP_004755979.1, XP_012913415.1, XP_012913416.1, and NP_077742.3, 3 ferret and 1 human isoforms of WT1, respectively²⁸). This high level of homology suggests that modulation of EMT or MET might play a role in the development of mesotheliomas in animals and supports the cross-species use of the anti-WT1 antibody reagent. Although WT1 expression is downregulated in many tissues after differentiation, the visceral epithelial cells (podocytes) of the mature glomerulus express WT1 throughout life,²⁹ making kidney a useful positive control tissue for WT1 immunohistochemistry.

Several histologic features have been used to differentiate mesothelioma from reactive benign mesothelium, as criteria for malignancy, and to determine prognosis in human mesothelioma. These features include histologic subtype, necrosis, mitotic count, nuclear and cytologic atypia, abundance of mesothelial proliferation, cellularity of papillary structures,

and others.^{30–32} Although data are not available to correlate histologic features of mesotheliomas with prognosis in ferrets, the tumor cells from the ferret of the present report expressed cytokeratin, vimentin, and the nuclear regulatory protein WT1, and the tumor encompassed the entire omentum and was comprised of densely cellular morular papillary structures and solid areas, with moderate nuclear atypia, foci of necrosis, intravascular tumor emboli, and metastatic spread to the diaphragm, consistent with malignancy. Usefulness of these histologic features to determine prognosis of mesothelioma in ferrets will rely on the accumulation of information from additional cases.

Acknowledgments

This work is supported in part by the National Institutes of Health through MD Anderson's Cancer Center Support Grant CA016672. The authors declare that there were no conflicts of interest.

References

1. Eguchi T, Kadota K, Mayor M, et al. Cancer antigen profiling for malignant pleural mesothelioma immunotherapy: expression and coexpression of mesothelin, cancer antigen 125, and Wilms tumor 1. *Oncotarget*. 2017;8:77872–77882.
2. Ordóñez NG, Sahin AA. Diagnostic utility of immunohistochemistry in distinguishing between epithelioid pleural mesotheliomas and breast carcinomas: a comparative study. *Hum Pathol*. 2014;45:1529–1540.
3. Blobel GA, Moll R, Franke WW, et al. The intermediate filament cytoskeleton of malignant mesotheliomas and its diagnostic significance. *Am J Pathol*. 1985;121:235–247.
4. García-Fadrique A, Mehta A, Mohamed F, et al. Clinical presentation, diagnosis, classification and management of peritoneal mesothelioma: a review. *J Gastrointest Oncol*. 2017;8:915–924.
5. Glickman LT, Domanski LM, Maguire TG, et al. Mesothelioma in pet dogs associated with exposure of their owners to asbestos. *Environ Res*. 1983;32:305–313.
6. Fels Elliott DR, Jones KD. Diagnosis of mesothelioma. *Surg Pathol Clin*. 2020;13:73–89.
7. Bibby AC, Maskell NA. Current treatments and trials in malignant pleural mesothelioma. *Clin Respir J*. 2018;12:2161–2169.
8. Katzman D, Stermann DH. Updates in the diagnosis and treatment of malignant pleural mesothelioma. *Curr Opin Pulm Med*. 2018;24:319–326.
9. Bueno R, Opitz I. State of the art review—surgery in malignant pleural mesothelioma. *J Thorac Oncol*. 2018;13. doi:10.1016/j.jtho.2018.08.001.
10. Cao S, Jin S, Cao J, et al. Advances in malignant peritoneal mesothelioma. *Int J Colorectal Dis*. 2015;30:1–10.
11. Batirel HF, Metintas M, Caglar HB, et al. Macroscopic complete resection is not associated with improved survival in patients with malignant pleural mesothelioma. *J Thorac Cardiovasc Surg*. 2018;155:2724–2733.
12. Son NV, Chambers JK, Shiga T, et al. Sarcomatoid mesothelioma of tunica vaginalis testis in the right scrotum of a dog. *J Vet Med Sci*. 2018;80:1125–1128.
13. Williams BH, Garner MM, Kawasaki TA. Peritoneal mesotheliomas in two ferrets (*Mustela putorius furo*). *J Zoo Wildl Med*. 1994;25:590–594.
14. Nakata M, Miwa Y, Nakayama H, et al. Localised radiotherapy for a ferret with possible anal sac apocrine adenocarcinoma. *J Small Anim Pract*. 2008;49:476–478.

15. Miwa Y, Kurosawa A, Ogawa H, et al. Neoplastic diseases in ferrets in Japan: a questionnaire study for 2000 to 2005. *J Vet Med Sci.* 2009;71:397–402.
16. Turner PV, Brash ML, Smith DA. *Pathology of Small Mammal Pets.* John Wiley & Sons Inc.; 2018.
17. Autieri CR, Miller CL, Scott KE, et al. Systemic coronaviral disease in 5 ferrets. *Comp Med.* 2015;65:508–516.
18. Mayer J, Marini RP, Fox JG. *Biology and diseases of ferrets.* In: Fox JG, Andersen LC, Otto GM, Pritchett-Corning KR, Whary MT, eds. *Laboratory Animal Medicine.* 3rd ed. Elsevier Science; 2015.
19. Quesenberry KE, Carpenter JW, Carpenter JW. *Ferrets, Rabbits, and Rodents: Clinical Medicine and Surgery.* Elsevier/Saunders; 2012.
20. Faig J, Howard S, Levine EA, et al. Changing pattern in malignant mesothelioma survival. *Transl Oncol.* 2015;8:35–39.
21. Harbison ML, Godleski JJ. Malignant mesothelioma in urban dogs. *Vet Pathol.* 1983;20:531–540.
22. Wolfe DF, Carson RL, Hudson RS, et al. Mesothelioma in cattle: eight cases (1970–1988). *J Am Vet Med Assoc.* 1991;199:486–491.
23. Damjanov I, Friedman MA. Mesotheliomas of tunica vaginalis testis of Fischer 344 (F344) rats treated with acrylamide: a light and electron microscopy study. *In Vivo.* 1998;12:495–502.
24. Mutsaers SE, Birnie K, Lansley S, et al. Mesothelial cells in tissue repair and fibrosis. *Front Pharmacol.* 2015;6:113.
25. Miller-Hodges E, Hohenstein P. WT1 in disease: shifting the epithelial-mesenchymal balance. *J Pathol.* 2012;226:229–240.
26. Menke AL, van der Eb AJ, Jochemsen AG. The Wilms' tumor 1 gene: oncogene or tumor suppressor gene? *Int Rev Cytol.* 1998;181:151–212.
27. Lee SB, Haber DA. Wilms tumor and the WT1 gene. *Exp Cell Res.* 2001;264:74–99.
28. National Center for Biotechnology Information (NCBI). BLAST: Basic Local Alignment Search Tool. Accessed Dec 19, 2020. <https://blast.ncbi.nlm.nih.gov/Blast.cgi>.
29. Guo JK, Menke AL, Gubler MC, et al. WT1 is a key regulator of podocyte function: reduced expression levels cause crescentic glomerulonephritis and mesangial sclerosis. *Hum Mol Genet.* 2002;11:651–659.
30. Pelosi G, Papotti M, Righi L, et al. Pathologic grading of malignant pleural mesothelioma: an evidence-based proposal. *J Thorac Oncol.* 2018;13:1750–1761.
31. Valente K, Blackham AU, Levine E, et al. A histomorphologic grading system that predicts overall survival in diffuse malignant peritoneal mesothelioma with epithelioid subtype. *Am J Surg Pathol.* 2016;40:1243–1248.
32. Kadota K, Suzuki K, Colovos C, et al. A nuclear grading system is a strong predictor of survival in epithelioid diffuse malignant pleural mesothelioma. *Mod Pathol.* 2012;25:260–271.