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Ten Cases of Feline Mesothelioma: an Immunohistochemical and Ultrastructural Study

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Summary

In the cat only 10 cases of mesothelioma, mainly of the peritoneum, have been previously reported. This paper describes a further 10 cases, eight pleural and two peritoneal, in males and females aged 1–17 years. Histologically, five tumours were epithelial, three fibrosarcomatous and two biphasic. Immunohistochemical markers used in human pathology for the identification of mesotheliomas include vimentin, cytokeratin (CK) AE1/AE3, HBME-1, CK 5/6, calretinin, thrombomodulin, carcinoembryonic antigen (CEA), CD15, E-cadherin and desmin. All 10 feline mesotheliomas were positive for vimentin and CK AE1/AE3, six were positive for HBME-1, two for CK5/6, three for CEA and four for E-cadherin. All were negative for desmin and calretinin. Antibodies to thrombomodulin and CD15 failed to cross-react with feline tissues. Electron microscopy, performed in four cases, revealed microvillar structures, desmosomes and intracytoplasmic lumina, confirming its value as a diagnostic tool. The study showed that mesothelial marker antibodies commonly used in human patients can be used for the diagnosis of feline mesothelioma, preferably as a panel of antibodies rather than only one.

Keywords: cat; epithelial mesothelioma; mesothelioma; pleural mesothelioma; tumour

Introduction

Mesotheliomas are malignant tumours that arise from the mesothelial cells of the pleura, peritoneum and pericardium, and occasionally from the tunica vaginalis testis. These tumours are rare in all species but have been recorded most frequently in man, expecially in association with exposure to asbestos (McCaughey *et al.*, 1983). In animals, spontaneously arising mesotheliomas have been reported most frequently in cattle and dogs, and less commonly in horses (Julian, 1993; Head *et al.*, 2002). In cattle, this neoplasm is often congenital, whereas it occurs in adult dogs (Head *et al.*, 2002). It rarely affects cats, only 10 cases having been reported (Andrews, 1973; Tilley *et al.*, 1975; Raflo and Nuernberger, 1978; Akiyama and Suzuki, 1982; Schaer and Mayer, 1988; Umphlet and Bertoy, 1988; Kobayashi *et al.*, 1994; Bertazzolo *et al.*, 2000; Granger *et al.*, 2003; Rinke and Rosenbruch, 2003). In animals, three main histological types are described: the epithelioid, the fibrosarcomatous (or fibrous), and the biphasic (or mixed) types (Head *et al.*, 2002).

Julian (1993) and Head *et al.* (2002) gave accounts of the macroscopical and histological features of feline mesothelioma, but there have been few investigations of immunohistochemical and ultrastructural characteristics. This paper describes the gross and histological findings as well as the immunohistochemical and ultrastructural features, in 10 cases of feline mesothelioma.

Materials and Methods

Samples

Ten samples (nos 1–10) originally identified as mesotheliomas were selected from the files of the Department of Veterinary Public Health and Animal Pathology, Alma Mater Studiorum, University of Bologna. These samples were collected during a 20-year period, from 1985 to 2004. Archived haematoxylin

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and eosin sections from samples fixed in Carson's formalin and embedded in paraffin wax were reviewed to re-assess the previous diagnosis of mesothelioma.

Tumour tissues were stained with Masson's trichrome to demonstrate the connective tissue component of the mesotheliomas. Lung tissue from each cat was evaluated for the presence of ferruginous bodies (suggestive of asbestos exposure) with Perls' Prussian blue stain.

Immunohistochemistry (IHC)

All mesotheliomas were labelled immunohistochemically with the following antibodies: vimentin, cytokeratin (CK) AE1/AE3, CK 5/6, HBME-1, CEA, E-cadherin, desmin and calretinin. Antibodies previously untested in the cat (CK 5/6, HBME-1, calretinin, thrombomodulin and CD15) were examined with feline tissues (Table 1), to ascertain cross-reactivity. Two of these antibodies (thrombomodulin and CD15) failed to cross-react and were therefore excluded from the study. IHC was performed with a commercial streptavidin–biotin peroxidase labelling kit (Dako, Glostrup, Denmark). Sections were dewaxed and rehydrated, and endogenous peroxidase was blocked by treatment with hydrogen peroxide 0·3% in methanol for 30 min. To unmask the antigen, sections were treated with citrate buffer, pH 6·00, 1 M (Merck, Darmstadt, Germany), or with ethylenediaminetetra-acetic acid buffer (EDTA), pH 8·00, 1 mM (Merck), by heating twice for 5 min in a microwave oven (750 W), and then being allowed to cool at room temperature for 20 min. HBME-1 did not require pretreatment. For carcinoembryonic antigen (CEA), endogenous peroxidase was blocked by incubation with hydrogen peroxide 3% in methanol for 30 min, and pretreatment was performed by trypsin digestion, pH 7·6, 0·1 M (Merck) at 37 °C for 20 min. Primary antibodies were diluted in phosphate-buffered saline (PBS) as indicated in Table 2 and incubated with the sections overnight at 4 °C in a moist chamber. Sections were then incubated with the secondary antibody (anti-rabbit IgG conjugated with biotin) for 15 min at room temperature and subsequently with the streptavidin-biotinylated peroxidase complex for 15 min at room temperature. The peroxidase reaction was developed with 0·04% diaminobenzidine (Sigma, Steinheim, Germany) in Tris for 10 min. Finally, the slides were counterstained with Papanicolau haematoxylin, dehydrated and mounted under DPX (Fluka, Riedel-de Haen, Germany). The positive controls for each run consisted of one section from a tissue known to express the antigen. As a negative control for non-specific binding of the secondary antibody, the primary antibody was replaced with an irrelevant, isotype-matched antibody.

Table 1
Cross-reactivity studies in feline tissues

Antibody	*Tissues tested*	*Cross-reactivity*
CK 5/6*	Skin	+(basal cells)
	Mammary gland	+(myoepithelial cells)
	Pleura	+(mesothelial cells)
HBME-1†	Pleura	+(mesothelial cells)
	Cartilage	+
Calretinin†	Brain	+(Purkinje cells)
	Testis	—
	Pleura	—
Calretinin‡	Brain	—
	Testis	—
	Pleura	—
Thrombomodulin†	Pleura	—
CD15†	Mammary gland	—
	Apocrine gland carcinoma	—

*Zymed, S. Francisco, USA.
†Dako, Glostrup, Denmark.
‡Novocastra, Newcastle upon Tyne, UK.

Table 2
Details of antibodies used for immunohistochemical examination of feline mesotheliomas

Antibody	*Supplier*	*Dilution*	*Pretreatment*	*Positive controls*
Vimentin*	Dako	100	C	Kidney
CK AE1/3*	Dako	100	C	Skin
CK 5/6†	Zymed	100	E	Skin
CEA*	Dako	200	T	Intestinal carcinoma
HBME-1*	Dako	100	—	Pleura
E-Cadherin‡	BD Transduction Labs	100	C	Mammary gland
Desmin*	Dako	50	C	Duodenum
Calretinin*	Dako	100	C	Brain

C, Microwave in citrate buffer; E, microwave in EDTA; T, trypsin digestion. All antibodies were monoclonal (mouse anti-human) except CEA (polyclonal, rabbit anti-human).
*Dako, Glostrup, Denmark.
†Zymed, S.Francisco, USA.
‡BD Transduction Laboratories, Lexington, USA.

Transmission Electron Microscopy (TEM)

This was performed on tissues from four cases (nos 2,3,4,9). Pieces of formalin-fixed tissue were rinsed twice in 0·1 M cacodylate buffer (pH 7·4), postfixed in 1% osmium tetroxide, and embedded in Araldite. Ultrathin sections were double-stained with uranyl acetate and lead citrate and examined with a Philips 410 electron microscope.