HISTOPATHOLOGICAL AND IMMUNOPHENOTYPICAL CHARACTERIZATION OF A COMBINED MELANOMA AND MUCOEPIDERMOID CARCINOMA IN A DOG

Simultaneous canine mucoepidermoid carcinoma and melanoma.

C.M.Martínez[1], A.J. Buendía[1], J.Sánchez[1], M. Vilafranca[2], J. Altimira[2], G. Ramírez[2], B. García[2] and J.A. Navarro[1].

[1]Departamento de Anatomía y Anatomía Patológica Comparadas, Facultad de Veterinaria, Universidad de Murcia, Campus de Espinardo, 30100 Espinardo, Murcia, Spain. and [2]Histovet S.L., Stz. Quirze del Vallés, Barcelona, Spain.

Autor para correspondencia: Carlos Manuel Martínez Cáceres. Teléfono: +34 868 88 4703; Fax: +34 868 88 4147. E-mail: cmmarti@um.es

SUMMARY

The present paper reports the first case of a canine high-grade (low differentiated) mucoepidermoid carcinoma, in which malignant proliferation of melanocytes has been detected. The tumoral mass was located in the lower neck, pressing against the 7th cervical vertebral body in an aged dog of mixed breed. No macroscopic signs of skin or oral melanoma or carcinoma were detected elsewhere. The microscopic examination showed close intermingling of the two components. Melanocytic proliferation rarely occurs accompanied by the colonization of nonmelanocytic tumors, and the cause of such proliferation remains unclear. In human pathology, several reports of pigmented mucoepidermoid carcinomas have been described, and in all cases the proliferation of the melanocytic component was considered as hyperplastic, and no sign of malignant proliferation was detected. However, neither in human nor in veterinary pathology has a case of intermingled melanoma and mucoepidermoid carcinoma been described.

Key words: canine, histopathology, immunohistochemistry, carcinoma, melanoma.

RESUMEN

El presente trabajo describe el primer caso de un carcinoma mucoepidermoide canino pobremente diferenciado en el que se ha observado una proliferación maligna de melanocitos. La masa tumoral se localizó a nivel del 7º cuerpo vertebral en perro de raza mestiza. No se detectaron otros signos macroscópicos de carcinoma y/o

melanoma oral o epitelial. El examen microscópico reveló la proliferación conjunta de ambas subpoblaciones. La proliferación y colonización de melanocitos en tumores de origen no melánico es rara y no se conoce la causa. En Patología humana, se han descrito casos de carcinomas mucoepidermoides pigmentados, pero en todos los casos se consideró la proliferación melanocítica como hiperplasia, no observándose en ningún caso signos de neoplasia. No existen en Patología humana ni veterinaria ningún caso descrito de carcinoma mucoepidermoide concomitante con un melanoma maligno, siendo éste el primer caso descrito.

Palabras clave: canino, histopatología, inmunohistoquímica, carcinoma, melanoma.

INTRODUCTION

Melanocytes are dendritic cells which originates from the neural crest. During embryonic development most melanocyte precursors migrate to the epidermis, hair follicles and orbital cavity, while a few reach other locations such as the dermis, leptomeninges, mucous membranes and certain visceras (Mooi and Krautsz 1992). Although these cells are normally inactive, in several circumstances of carcinogenesis these pre-existing melanocytes are stimulated to produce melanin and proliferate to form non-neoplastic hyperplasia. This fact has been reported not only in some epithelial tumors of mucocutaneos origin, like squamous cell carcinomas (Bleehem 1975) or eccrine gland tumors (Wilson-Jones 1971), but also in a few extramucocutaneous epithelial tumors, such as thymic (Lagrange *et al.* 1987) and prostatic (Furusato *et al.* 1989) carcinomas.

Mucoepidermoid carcinoma is a malignant mucocutaneous epithelial tumor composed of a mixture of squamous cells, mucous-producing cells and intermediate (clear) type cells. A review of the literature yielded only a few documented cases in human pathology of pigmented mucoepidermoid carcinoma with a stromal non-neoplastic melanocytic hyperplasia (Mooi and Krautsz 1992, Takeda and Kurose 2006). In Veterinary pathology there are few reports describing canine mucoepidermoid carcinomas (Karbe and Schiefer 1967, Confer and DePaoli 1978) without signs of melanocytic hyperplasia. On the other hand, canine melanoma is a relatively common malignant neoplasia accounting for 3% of all neoplasms and up to 7% of malignant tumors (Cotchin 1955). The most frequency affected sites are the oral cavity (56%), lip (23%), skin (11%), and digit (8%), with other sites including the eye, accounting for only 2% (Goldschmidt and Shofer 1992). This paper describes one case of a combined canine high-grade mucoepidermoid carcinoma and malignant melanoma which showed close intermingling of the two components, a pathological finding not previously described in the literature.

MATERIAL AND METHODS

This report describes the case of a 15 year old male mixed-breed dog with a severe lameness in the front-left extremity. Clinical examination revealed a deep pain on the left side of the lower neck with severe loss of motor and cutaneous sensitivity of the extremity. No abnormal parameters either in blood biochemistry or hematology were detected. A mielographic examination detected a filling defect located at the 7th cervical vertebra, while the nuclear magnetic resonance (NMR) showed a mass pressing against the vertebral body. No other abnormalities were detected by NMR. Due to the lack of motor and cutaneous sensitivity, the surgeon decided to amputate the extremity. During surgery, the surgeon observed that the mass extended to the braquial plexus, pressing the nerve. After surgery, the animal´s health gradually deteriorated and, in view of the poor prognosis and the absence of a specific therapy, the owner elected euthanasia.

Macroscopically, the mass detected by NMR was movable, non-encapsulated and slightly

Table 1. Antibodies used in this study

Antibody	Clone	Isotype	Dilution	Pretreatment
Anti- Cytokeratin	MNF116	IgG1	1:50	Pronase-RT
Anti-Vimentin	V9	IgG1	1:50	Citrate buffer pH 6.0-121ºC
Anti-melan-A	A103	IgG1	1:50	EDTA pH 8.0-121ºC
Anti-S100 protein	Polyclonal	-	1:250	Citrate buffer pH 6.0-121ºC
Anti-glial fibrillary acid protein (GFAP)	Polyclonal	-	1:250	None
Anti-Neurofilament	NR4	IgG1	1:50	Citrate buffer pH 6.0-121ºC
Anti-Ki-67 protein	MIB-1	IgG1	1:100	Citrate buffer pH 6.0-121ºC

compressive. No other abnormalities were detected by the clinician, so a probable nervous tumor was considered as the primary diagnosis. Samples of the mass were then collected and fixed in 10% buffered formalin, embedded in paraffin wax and sectioned (4µm). For histopathological examination, the sections were stained with haematoxylin and eosin (HE), periodic acid-Schiff (PAS), alcian blue (AB). The immunohistochemistry study was carried out by the avidin-biotin-peroxidase complex (ABC) whith several primary antibodies whose clone and pretreatment for antigen retrieval are summarized in Table 1. All antibodies were purchased from Dako Corp. (Carpinteria, CA). Briefly, after antigen retrieval, samples were incubated in a methanol and 0.03% hydrogen peroxide solution (Panreac Química, Barcelona, Spain) for endogenous peroxidase block. Sections were incubated with normal serum (Vector Laboratories, Burlingham, UK) for 20 minutes and then incubated for 1 hour at 37ºC with the primary antibody. After washing with PBS, sections were then incubated for 20 minutes with the secondary antibody (Dako, Carpinteria, USA) and after washing in PBS, with the ABC complex (Vector Labs). Immunoreaction was revealed with 3-3´-Diaminobencidine (Dako Corp). Finally, sections were counterstained with Mayer's haematoxylin. Positive and negative control sections were included for each antibody.

RESULTS AND DISCUSSION

Microscopically, the mass compressed the spinal cord, but not invade the nervous tissue. A detailled histopathological and immunohistochemical examination revealed two different neoplastic proliferations (Figure 1). The first neoplastic population was composed of anaplastic and pleomorphic cells variable in size with acidophilic and vaculoated cytoplasm and a large round nucleus with a well developed eccentric nucleoli, arranged in dense solid masses, irregular nests and cystic or tubuloacinar structures whith a high mitotic rate. The predominat epithelial cell population was composed by cells which showed epidermoid squamous differentiation (poligonal cells with an abundant eosinophilic cytoplasm, central euchromatic nucleus with a well developed central nucleolus, arranged in nests, occasionally with keratin perl formation) and interspersed among this population several nests with clear-cytoplasm cells (small round to oval cells with a large round nucleus, eccentrical nucleoli and abundant pale eosinophilic cytoplasm) and scanty mucous-producing-cells (large round cells with a round nucleus and centric well developed nucleoli which containing intracytoplasmatic PAS and AB-positive vacuoles) were also observed. Immunohistochemically, these cells populations showed a strong immunoreaction against anti-cytokeratin antibody (Figure 2). Taken together,

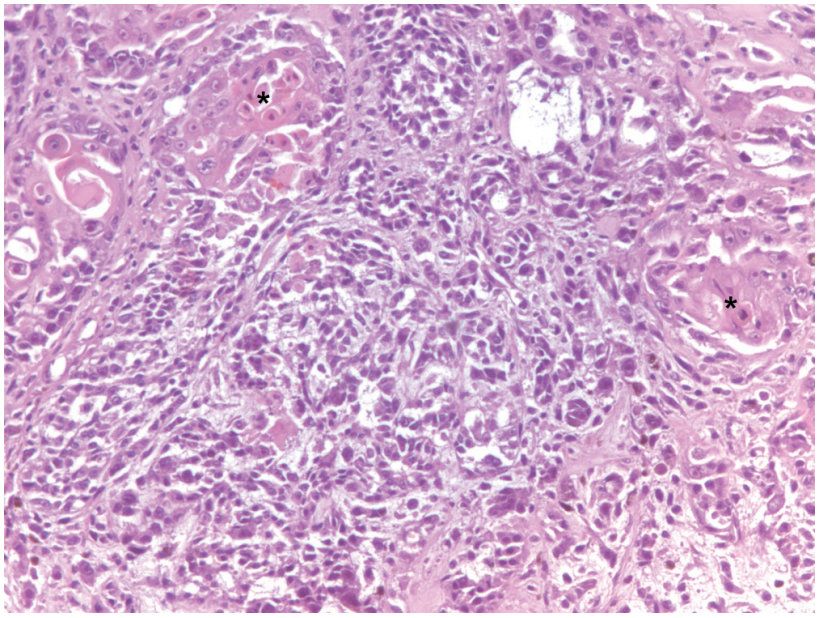

Figure 1: Microscopic image of the tumoral mass; cells with acidophilic and vacuolated cytoplasm, some of them with epidermoid squamous differentiation (asterisk) form irregular nests and tubuloacinar structures. Haematoxilyn and eosin. x200

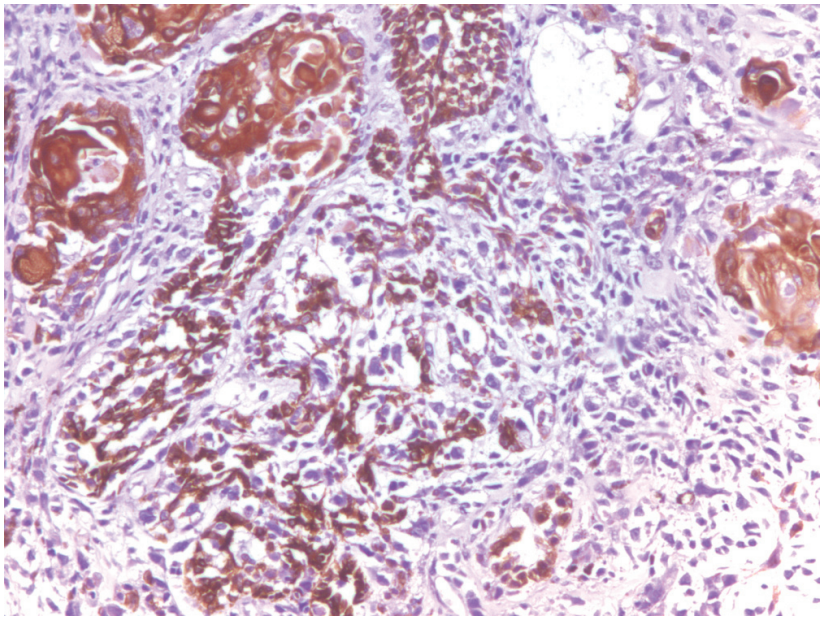

Figure 2: Expression of cytokeratin of the same zone of the Fig 1. There are non positive cells interspersed among positive cells. Avidin-biotin peroxidase against cytokeratin. X200

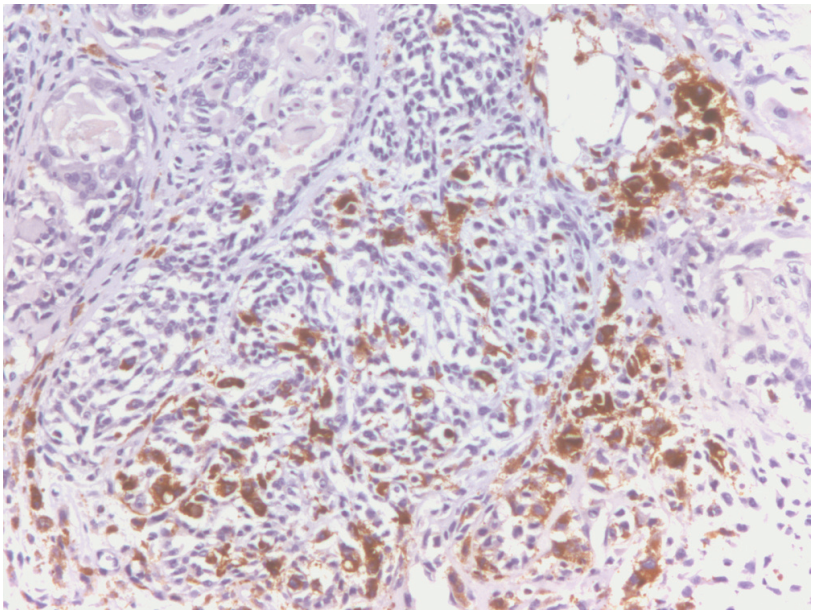

Figure 3: Expression of melan-A antigen of the same zone of the Fig 1. Note that positive cells are interspersed with cytokeratin-positive cells (see Fig 2). Avidin-biotin peroxidase against melan-A antigen. X200

these findings pointed to histological features of a mucoepidermoid carcinoma. A review of the literature showed that the histological grading of mucoepidermoid carcinoma remains controversial, but it is suggested that anaplastic cells and mitotic figures are tipically observed in high grade mucoepidermoid carcinomas. By the other hand, less than 10% of neoplastic cells are mucous-producing cells, which often cannot be readily identified without specific stains (Auclair *et al.* 1992). Taken together, these findings are compatible with a high-grade mucoepidermoid carcinoma.

To the best of our knowledge all reported cases of canine mucoepidermoid carcinoma locates the primary neoplasm in the salivary glands (Head and Else, 2002) and nasal cavity (Confer and DePaoli 1978), but none has been described in the lower neck. Although the mass was first considered to be a probable metastatic focus of a distant primary tumor located in some glandular region near the neck, perhaps the salivary or thyroid glands, no signs of abnormal growth were detected in these regions during clinical examination, NMR or during necropsy. In human pathology primary mucoepidermoid carcinomas have also been described in mammary gland (Hornychová *et al.* 2007), pancreatic gland (Onoda *et al.* 1995), thyroid gland (Franças *et al.* 2006) even in cervical lymph nodes (Sáez Santamaría *et al.* 2003) and upper and lower neck regions (Daniel and McGuirt 2005) where heterotopic normal salivary tissue have been described (Ferlito *et al.* 1999). Although the presence of heterotopic tissue has not been studied in dog, the primary neoplasm could be originated from some heterotopic glandular tissue locate in lower neck which, and due to its aggressive behaivour could spread to the brachial plexus.

A second neoplastic proliferation was observed interspersed the fibrovascular stroma of

the mucoepidermoid carcinoma. This second neoplasia was composed of anaplastic and pleomorphic cells with a fusiform or epithelioid in shape (predominant fusiform), round to oval nucleus with a one or two large and eccentrical nucleoli and showing strong immunoreaction against the anti-vimentin antibody. Several granules containing brown pigment were observed within the cytoplasm of some of these cells which could be removed using both hydrogen peroxide and potassium permanganate solutions, so this pigment was identified as melanin. Immunocytochemically, these cells expressed S-100 protein and melan-A (Figure 3), a typical melanocytic pattern. Although the staining with S-100 protein was stronger than melan-A, this latter immunomarker is considered a more specific and sensitive marker for diagnostic canine melanocytic neoplasias (Ramos-Vara *et al.* 2000). No inmunoreaction against GFAP or neurofilaments was observed. Taking together these features, the primary diagnostic of this second neoplasm was melanoma.

To evaluate the grade of malignancy of the melanocytic neoplasia, several parameters recommended by the World Health Organization (WHO) (Goldschmidt *et al.* 1998) were analized: cytological features (grade of atypia, variation in nuclear size and shape, presence of large nucleus, hyperchromasia, number and situation of nucleoli) and mitotic index (MI), calculated as the average number of mitoses per field after observation of ten high-power fields (hpf) (x400). The proliferation index (PI) was also evaluated by measuring the percentage of positive nuclei of the proliferation marker Ki-67 in 600 neoplastic melanocytic cells, since increased expression of the marker has been associated with malignancy in melanomas (Laprie *et al.* 2001, Millanta *et al.* 2002). Melanocytic neoplastic cells showed marked atypia, with a large nucleus with variable shape (round or fusiform) and size, accompanied by one or more eccentrical nucleoli. The MI was average (+/- 4.13 per hpf) with a PI of 45%. These results pointed to a malignant melanoma.

To our knowledge, no reports of a tumor with these two malignant components have been published in veterinary or human pathology. Few reports have been described pigmented mucoepidermoid carcinomas in humans (Marucci *et al.* 2005, Takeda and Kurose 2006), but this pigmentation was due to the proliferation and colonization of non-neoplastic melanocytic cells which produced granules of melanin, which were probably phagocytosed by neoplastic epithelial cells in a similar way to that known to occur with epidermal melanocytes (Marucci *et al.* 2005). In our case, the epithelial component was not pigmented, and only scattered melanocytes presented intracytoplasmatic melanin granules.

The cause of this malignant melanocytic proliferation is not clear, although some theories may be proposed. The mass was located in the lower neck, so the melanocytic component could be a part of an infiltrative melanoma originating in the skin which infiltrates the mucoepidermoid carcinoma. However, this does not seem to be likely because no signs of skin or oral melanocytic neoplasia were detected during clinical examination or necropsy.

By the other hand, inactive melanocytes have been detected in the oral mucosa and normal human parotid and minor salivary glands (Takeda 1997, 2000), so proliferation and malignization of a pre-existing melanocyte population initially located in the gland where the primary tumor was located is a second hypothesis to be considered, because=under certain conditions of tumorigenic transformation or growth of epithelial cells, these melanocytes could become activated, proliferate and produce melanin pigment (Takeda and Kurose 2006). A third hypothesis suggest the existence of a migration factor from cancer cells which would act as a stimulating factor for skin melanocyte migration, proliferation and melanin production (Azzopardi and Eusebi 1977).

Although several reports describe canine mucoepidermoid carcinoma this is the first to describe this tumor with a melanocytic malignant component. The phenomenon of malignant melanocytic proliferation in non-melanocytic tumors should be taken in to consideration in cases of pigmented lesions and the possibility should be evaluated for a good tumor prognosis.

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