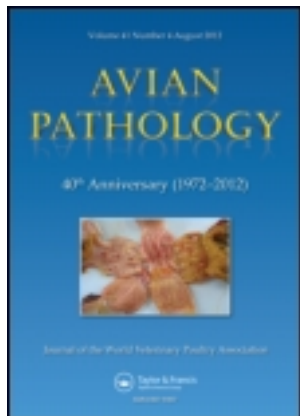


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Histopathologic, immunohistochemical and ultrastructural features of a granular cell tumour in an Australian parakeet (*Melopsittacus undulatus*)

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An adult male Australian parakeet (*Melopsittacus undulatus*) presented a firm nodular lesion in the lateral metacarpal region of the right wing. Microscopically, there were neoplastic cells, round and polyhedral in shape, with abundant, slightly eosinophilic granular cytoplasm; they were strongly periodic-acid Schiff-positive and resistant to diastase digestion. Some groups of neoplastic cells were immunopositive for smooth muscle actin and desmin. There was no immunopositivity for S-100 protein, CD68 and cytokeratin. Ultrastructurally, the neoplastic cells were round and polygonal in shape, and they were characterized by abundant cytoplasm with numerous homogeneous osmophilic bodies covered by an electron-dense membrane (lysosomes). The histopathologic, immunohistochemical and ultrastructural features of the neoplastic tissue are consistent with a granular cell tumour, which has been described in different animal species and anatomic locations; however, this seems to be an infrequent neoplasm in Australian parakeets. The immunopositivity of the neoplastic cells for smooth muscle actin and desmin, as well as slight positivity for muscle with Masson's trichrome, suggest that this is a tumour of myogenic origin.

Introduction

Neoplasms represent 3 to 4% of the histopathologic lesions in psittacines, of which two-thirds are described in Australian parakeets (*Melopsittacus undulatus*). This is also considered to be the psittacine with the greatest predisposition to develop neoplastic lesions (Alistair, 2005). The neoplasms that have been described frequently in birds are lipomas, adenomas and renal adenocarcinomas, and testicular, ovarian, liver and thyroid neoplasms (Suchy *et al.*, 1999; Alistair, 2005). The granular cell tumour is an infrequent neoplasm; it has been described relatively frequently in dogs, horses, cats (Patnaik, 1993), humans (Luaces *et al.*, 2007), rats and mice (Courtney *et al.*, 1992; Veit *et al.*, 2008), but only two cases appear to have been described in birds (Patnaik, 1993; Quist *et al.*, 1999). Although it was first described in 1926 by Abrikossoff, the histogenesis of this neoplasm has not been determined thoroughly (Budiño *et al.*, 2003; Luaces *et al.*, 2007). According to immunohistochemical studies, and depending on the species in which it is present, it has been theorized that the tumour could be muscular, neural, epithelial or histiocytic in origin (Patnaik, 1993; Kelley *et al.*, 1995; Veit *et al.*, 2008). Other studies suggest that the granular cell tumour is an expression of degenerative changes that may occur in diverse types of cell (Alidina *et al.*, 1994). The aim of this work was to describe the histopathologic

(morphology, histochemistry), immunohistochemical and ultrastructural features of a granular cell tumour diagnosed in the skin of an Australian parakeet.

Materials and Methods

Case history. An adult male Australian parakeet was submitted for physical examination because of the presence of a tumour-like lesion in the lateral metacarpal region of the right wing of unknown duration. Seven months before, surgery had been performed in the same region, during which a tumour had been removed incompletely, but it was not sent for histopathologic examination. At physical examination, the patient was alert and responsive, and weighed 35 g. The bird lacked feathers in the metacarpal region and presented a 1.5 × 1.0 cm² nodular lesion, which was attached firmly to the bone. The parakeet was presented to the hospital for surgical removal of the mass.

Histopathology. The tissue was fixed in 10% buffered formalin for 24 h and a routine histological technique was performed. Sections were cut with a thickness of 3 µm and stained with haematoxylin and eosin, periodic-acid Schiff (PAS), PAS diastase, phosphotungstic acid haematoxylin, Ziehl–Neelsen and Masson trichrome stains.

Immunohistochemistry. Samples of neoplastic tissue were sent to the Pathology Unit of the General Hospital in Mexico. Immunohistochemistry was performed by the labelled streptavidin–biotin–peroxidase

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complex technique. A panel of primary antibodies against desmin (muscle), smooth muscle actin (muscle), protein S-100 (neural), cytokeratin (epithelial) and CD68 (macrophages) was used (Dako North America, Carpinteria, California, USA) (Table 1). Goat anti-mouse, anti-rabbit biotinylated secondary antibodies were applied and diaminobenzidine tetrahydrochloride was used as the chromogen. Antigen retrieval for all antibodies was performed with citrate buffer, pH 9, in a pressure cooker.

Electron microscopy. From the tissue obtained by biopsy, fragments of 3 mm² were fixed in glutaraldehyde at 2.5%, fixed with osmium tetroxide at 1% for 2 h and washed with 0.1 M cacodylate buffered solution (pH 7.2). Subsequently, they were dehydrated in acetone solutions of increasing concentration, embedded in epoxy resins (Epon 812, Electron Microscopy Sciences, Industry Road Hatfield, PA), and finally polymerized at 60°C for 24 h. Afterwards, semi-thin cuts of 200 µm were made using an ultramicrotome, and the sections were mounted on slides, which were contrasted with toluidine blue (Hayat, 2000). Thin cuts of 60 µm were made, mounted on a copper mesh, contrasted finally with uranyl acetate and lead citrate, and observed under an electron microscope (Zeiss EM-900, Zeiss, Oberkochen, Germany) at 80 kV.

Results and Discussion

The neoplasm measured 1.5 × 1.0 × 0.5 cm³ with well-demarcated borders and was firm to the touch. The cut surface was smooth and white. Microscopically, neoplastic tissue was observed in the dermis and was characterized by hypercellular areas composed of round and polyhedral-shaped neoplastic cells arranged in a solid pattern. The neoplastic cells showed abundant slightly eosinophilic cytoplasm with well-defined borders and with abundant granules in the interior. The nucleus was round to oval-shaped, euchromatic with fine granular chromatin and marked anisokaryosis. There were scarce mitotic figures (zero to two per random field with a 40× magnification lens). In other zones, large neoplastic cells and multi-nucleated giant cells were observed. The tumour was unencapsulated and the granular cells infiltrated surgical margins (Figure 1).

The intracytoplasmic granules were magenta-coloured with PAS stain and they were resistant to diastase digestion, which ruled out the presence of glycogen in the cytoplasm of the neoplastic cells; no striations were observed with phosphotungstic acid haematoxylin stain. The neoplastic cells were light pink-coloured with Masson's trichrome stain, and the Ziehl-Neelsen stain was negative.

Some groups of neoplastic cells showed immunopositivity for smooth muscle actin and desmin. There was no immunopositivity for S-100 protein, CD68 and cytokeratin (Figure 2).

Ultrastructurally, the neoplastic cells were round to polygonal in shape, and were characterized by abundant

Table 1. Details of primary antibodies used for immunohistochemistry.

Antibody ^a	Origin	Reactivity	Dilution
Smooth muscle actin	Mouse	Muscle	1:400
Desmin	Mouse	Muscle	1:100
Protein S-100	Rabbit	Neural	1:200
Cytokeratin	Mouse	Epithelial cells	1:50
CD68	Mouse	Macrophages	1:1600

^aAll products were obtained from Dako North America (Carpinteria, California, USA).

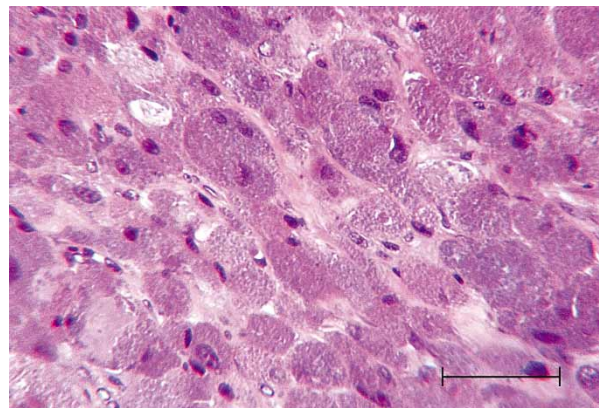


Figure 1. Skin of an Australian parakeet (*M. undulatus*). The dermis shows hypercellular areas composed of rounded neoplastic cells, with a small eccentric nucleus and abundant cytoplasm with well-defined borders and abundant granules in its interior. Haematoxylin and eosin. Bar = 100 µm.

cytoplasm with slightly electron-dense fibrillar material and numerous free ribosomes. The cytoplasm showed numerous homogeneous osmophilic bodies covered by an electron-dense membrane (primary and secondary lysosomes), as well as autophagosomes. The nuclei were round to oval in shape; some were indented and displaced to the periphery of the cell, with a wavy nuclear membrane of irregular border. Some nuclei showed abundant disperse euchromatin and prominent central nucleoli (Figure 3).

The histological (morphology and histochemistry) and ultrastructural features were consistent with a granular cell tumour, which has been described in different animal species and anatomic locations but always with similar morphological characteristics. The tumour has been diagnosed mainly in dogs and horses (Patnaik, 1993). In humans and dogs, this tumour is located mainly in the tongue (Barnhart *et al.*, 2001; Luaces *et al.*, 2007; Rossi *et al.*, 2007) and it is believed that it has a histiocytic origin (Patnaik, 1993). In horses, the granular cell tumour has been reported only in the lung, and it is thought to originate from Schwann cells (Kelley *et al.*, 1995). In rats and mice, this tumour has been reported in the female reproductive tract (Courtney *et al.*, 1992; Veit *et al.*, 2008). In these rodents, it has

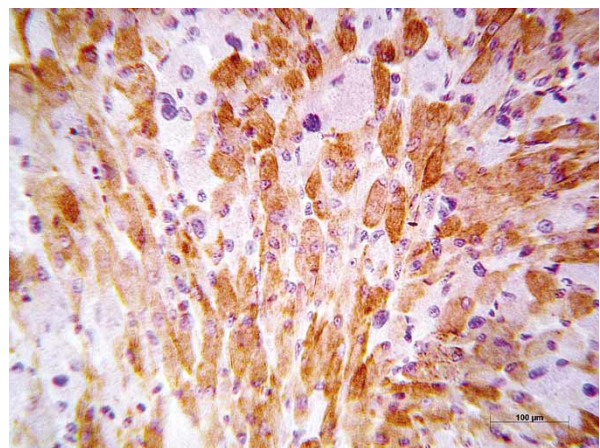


Figure 2. Immunohistochemistry of the skin of an Australian parakeet (*M. undulatus*). The cytoplasm of some groups of neoplastic cells is strongly positive for desmin. Bar = 100 µm.

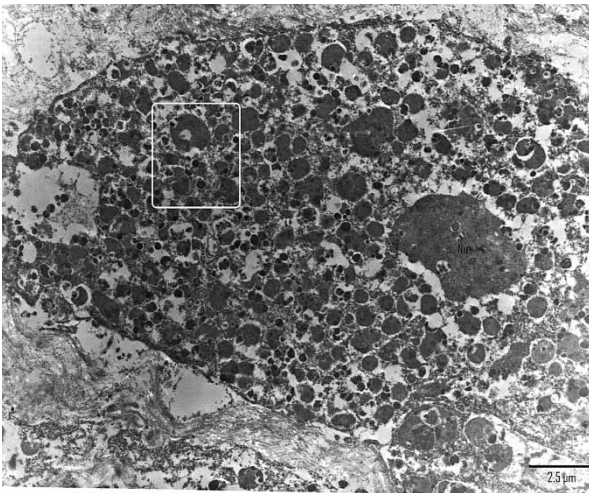


Figure 3. Electron microscopy of the skin of an Australian parakeet (*M. undulatus*). Note the numerous primary and secondary lysosomes (autophagosomes) of different diameters in the neoplastic cell cytoplasm (box). Next to the cell there are collagen bands (*). Nu, nucleus. General contrast technique with uranyl acetate and lead citrate. Bar = 2.5 μ m.

been observed that the cellular composition, morphology, and immunohistochemical staining profile of granulated metrial gland cells are similar to reported granular cell tumours. The granulated metrial gland cells are bone-marrow-derived, perforin-positive, natural killer cells that proliferated in the pregnant uterus (Picut *et al.*, 2009). However, it seems that this neoplasm is infrequent in birds, because there have been only two reported cases. The first was reported in a cockatiel (*Nymphicus hollandicus*), which presented a 0.5 cm nodular lesion in the right periorbital region. The neoplastic cells were round to oval-shaped with granular cytoplasm, and the granules were PAS-positive and resistant to diastase digestion (Patnaik, 1993). The second case was reported in a Puerto Rican parrot (*Amazona vittata*), which presented a nodule 3 cm in diameter in the left metacarpal region. The neoplastic cells were round to polygonal with abundant, slightly eosinophilic cytoplasm and with numerous granules in their interior, which were PAS-positive and diastase resistant. Ultrastructurally, numerous mitochondria and lysosomes were observed, similar to the ones observed in the case reported here (Quist *et al.*, 1999). Patnaik (1993) reported immunopositivity for desmin and actin and Quist *et al.* (1999) mentioned a positive reaction for desmin and vimentin. This is consistent with the results reported in the present paper, where positivity of neoplastic cells for smooth muscle actin and desmin was found, which suggests that granular cell tumours in the skin of psittacines may have a myogenic origin. The immunopositivity for vimentin is irrelevant because it is found in cells of mesenchymal origin and expressed in some carcinomas; therefore, it is generally considered very nonspecific and unreliable. The present case showed slight positivity for muscle with Masson's trichromic stain. Muscular origin has also been suggested for granular cell tumours described in the uteri of rats (Veit *et al.*, 2008), the toe of a cat (Patnaik, 1993) and the tongue of a dog (Rallis *et al.*, 2001).

The microscopic characteristics suggest benign biological behaviour. This is consistent with the current clinical profile, because the patient has been examined periodically and during the physical examination the specimen is responsive, has not lost weight (body weight 35 g) and there is no macroscopic evidence of tumour relapse. This is consistent with the literature (Patnaik, 1993; Budiño *et al.*, 2003; Luaces *et al.*, 2007; Rossi *et al.*, 2007); however, in humans and some other animals (Khansur *et al.*, 1987; Patnaik, 1993; Budiño *et al.*, 2003) malignant variations of these tumours are characterized by locally aggressive behaviour—they can be present in critical anatomic locations or show distant metastasis. In the medical literature reports that are based on the benign biological behaviour of the majority of granular cell tumours, the treatment of choice in humans and domestic animals is surgery (Budiño *et al.*, 2003; Reavill, 2004; Luaces *et al.*, 2007).

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