Disease in Wildlife or Exotic Species

SHORT TITLE: Scent Gland Adenocarcinoma in a Capuchin Monkey

Short Paper

Scent (apocrine) gland adenocarcinoma in a wedge-capped capuchin monkey (*Cebus olivaceus*): Histological and Immunohistochemical Features

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Summary

In human beings, apocrine gland tumours encompass a heterogeneous group of uncommon neoplasms with varied and unpredictable biological behaviour. They can be slow-growing lesions, recur after excision, produce lymph node metastasis in up to 50% of cases or lead to tumour-related death. We document a malignant scent adenocarcinoma in a wedge-capped capuchin monkey (*Cebus olivaceus*). Immunohistochemical labelling revealed complete absence of myoepithelial cells, a finding usually considered a hallmark of malignancy in human beings; however, after two-year follow-up, the neoplasm had not recurred. This is the first detailed report of the pathology of a spontaneous scent (apocrine) gland adenocarcinoma in a nonhuman primate.

***Keywords*:** adenocarcinoma; apocrine gland; non-human primate; scent gland

New world monkeys (NWM), order Platyrrhini, include the Aotidae, Pitheciidae, Atelidae and Cebidae families. The Cebidae encompass the Callitrichinae (tamarins and marmosets), Saimiriinae (squirrel monkeys) and Cebinae (capuchin monkeys) subfamilies. Capuchin monkeys include the genera Sapajus (robust capuchins) and Cebus (gracile capuchins). Capuchins are distributed throughout Central and South America, although, their motor skills, behavioural and feeding pattern flexibility and aptitude for problem solving enable them to adapt to a wide range of habitats (Wynne *et al*., 2004; Fedigan, 2017). Several capuchin species are listed as Critically Endangered or Endangered on the 2020 IUCN Red List (https://www.iucnredlist.org/, accessed 6th July 2020) with destruction of their habitat the major recognized threat. Furthermore, their extrovert behaviour and well-developed manual skills, have unfortunately made them a target for the illegal animal trade, and they have been referred to as "organ-grinder monkeys" (Lynch Alfaro *et al*., 2014; Fedigan, 2017).

Spontaneous neoplasms of capuchin monkeys have been only rarely reported (Cameron and Conroy, 1976; Brown *et al*., 1980; Grana *et al*., 1992; Klinger *et al*., 1993; Kramer and Bielitzki, 2012) and are mainly squamous cells carcinomas in the oral cavity or haired skin. Adnexal tumours are much less common and include trichoepithelioma and sebaceous gland adenoma (Kramer and Bielitzki, 2012).

Sweat glands (eccrine and apocrine) play an important role in non-human primate (NHP) behaviour. Although perspiration is not the major thermoregulatory mechanism in NHP, eccrine sweat glands are present throughout the body. Apocrine glands, frequently referred to as "scent glands", dominate chemical signalling and are located in the perineal, pubic and sternal regions (Perkins and Ford, 1969; Matz-Rensing and Lowenstine, 2018). Sweat gland tumours in human beings encompass a heterogeneous group of neoplasms of varied biological behaviour, ranging from benign to highly malignant (Cardoso and Calonje, 2015). Proliferative and neoplastic sweat gland lesions in dogs and cats are frequent and can be categorized as

simple or complex according to the presence of luminal secretory epithelium only or both luminal secretory epithelium and a myoepithelial layer, respectively (Gross *et al*., 2005).

Only one case of apocrine gland adenocarcinoma (AAC) in a NHP (white-fronted capuchin monkey; *Cebus albifrons*) has been described (Cameron and Conroy, 1976). Apart from that brief report, detailed histological and immunohistochemical descriptions of a scent gland carcinoma in a NHP have not been published. This report describes the gross, microscopic and immunohistochemical features of an apocrine (scent) gland adenocarcinoma with a two-year follow-up in a wedge-capped capuchin (*Cebus olivaceus*).

Clinical examination of a 25-year-old, male, wedge-capped capuchin monkey, with a 2-week history of pectoral swelling revealed a 3 x 3 x 1.5 cm, firm, cutaneous mass with locally extensive ulceration (Fig. 1) but no evidence of axillary lymphadenomegaly. Surgical excision was performed under general anaesthesia and on cut surface, the mass was dark-red and gritty. The excised mass was fixed in 10% neutral buffered formalin and submitted to the Pathology & Diagnostic Laboratories at the Royal Veterinary College. Representative sections were trimmed, processed routinely and embedded in paraffin-wax and sections cut (4 μm) and stained with haematoxylin and eosin (HE).

Microscopically the dermis was locally and extensively expanded by a well-demarcated but focally infiltrative, unencapsulated, densely cellular, epithelial neoplasm (Fig. 2). Neoplastic cells were arranged in 1 to 3 cell layer-thick tubules, with a narrow lumen that blended with multifocal solid areas. Tubules were supported by cores of dense fibrovascular stroma and multifocal areas of coagulative necrosis representing 5-10 % of the neoplasm. There were multifocal lakes and cords of hyaline stroma (collagenous spherulosis) (Fig. 3 and Supplementary Fig. 1). Multifocally the superficial dermis was infiltrated by trabeculae, nests and discohesive clusters of neoplastic cells with distinct cuboidal to polyhedral borders and moderate amounts of brightly eosinophilic, occasionally vacuolated cytoplasm. Decapitation

secretion (apocrine blebbing) was evident. Neoplastic cells frequently contained one large, eccentric, pleomorphic nucleus with coarsely stippled chromatin and one prominent hyperchromatic nucleolus. Nuclear pseudoinclusions, binucleated and trinucleated cells and macrokaryosis were frequent. Anisocytosis and anisokaryosis were moderate to marked and 13 mitotic figures were counted in 10 high-power-fields (400x). The neoplasm was surrounded by multiple lobular units of apocrine glands exhibiting occasional dysplastic changes, including loss of nuclear orientation and bizarre hyperchromatic nuclei. The overlying epidermis was extensively ulcerated and covered by a thick sero-cellular crust containing colonies of Gram-positive coccoid bacteria. Areas of intact epidermis had regular acanthosis and the underlying dermis was diffusely oedematous and multifocally infiltrated by lymphocytes, plasma cells and fewer neutrophils. Vascular or lymphatic invasion was not observed and the lateral and deep surgical margins (3mm width) were free of neoplastic cells. Based on the presence of areas of infiltration, solid growth patterns, moderate to marked anisocytosis, anisokaryosis, nuclear pleomorphism and a high mitotic rate, a diagnosis of adenocarcinoma was reached.

Immunohistochemistry (IHC) was performed using 3µm thick serial sections mounted on positively charged glass slides and a panel of antibodies against high molecular weight cytokeratins and other cellular proteins (Table 1). Antigen-antibody reactions were visualized with the Bond Polymer Refine Detection kit (Leica, Milton Keynes, UK). The proliferation index (PI) was calculated by counting the nuclei positive for Ki67 antigen in 1,000 neoplastic cells. Human and canine skin, mammary gland and lung squamous cell carcinoma tissues were used as positive controls. As negative controls, primary antibodies were replaced by homologous non-immune serum (Suarez-Bonnet *et al*., 2017).

Supplementary Table 1 summarizes the immunohistochemical observations made in normal and neoplastic apocrine gland tissue. In both normal and neoplastic tissue, luminal epithelial cells labelled intensely for E-Cadherin, AE1/AE3, CAM5.2, CK7 and CK8 antigens (Fig. 3).

The antibodies against 34βE12, CK5/6 and CK14 intensely labelled myoepithelial cells in the peripheral, non-neoplastic apocrine acini (Fig. 4). However, in areas with dysplastic changes there was discontinuity and gaps in the continuous layer of myoepithelial cells surrounding the luminal epithelium. In contrast, there was no evidence of myoepithelial cells within the apocrine neoplasm. Ki67 labelled the stratum basale of the epidermis, hair follicles and sebaceous reserve cells. Ki67 was detected in the nuclei of 12% of the neoplastic cells. Anti-CK19, CK20, ORα, PR or CEA antibodies did not label normal or neoplastic tissue. All tissue controls labelled appropriately. Based on the gross, microscopical findings and the immunohistochemical profile, a diagnosis of simple apocrine (scent gland) adenocarcinoma was established. To the authors' knowledge, this is the first comprehensive report of the gross, histopathological and immunohistochemical features of normal, dysplastic and neoplastic apocrine (scent) gland tissue in a non-human primate.

Microscopical differentiation between apocrine and eccrine glands can be challenging and some authors have claimed that unequivocal distinction is not possible using histology alone (Gross et al., 2015). However, a histological hallmark of apocrine glands, not observed in either normal or neoplastic eccrine neoplasms, is the formation of cytoplasmic blebs (aposomes) which consist of a network of cytoskeleton and serum albumin (Gross et al., 2005; Santa Cruz, 2007; Miyamoto *et al*., 2009). In dogs and cats, apocrine glands are distributed throughout the body including the anal sacs and the perianal and footpad zones. The latter is the only area in which eccrine glands are located in these species (Gross et al., 2005). In both human beings and NHP, apocrine glands are densely located in the anogenital region, axilla, eyelid (Moll's gland) and external ear canal. Solitary apocrine units can be found to a much lesser extent, probably for evolutionary reasons, in the breast region, lip, hand and foot (Cardoso and Calonje, 2015; Matz-Rensing and Lowenstine 2018). In contrast, NHP have numerous apocrine glands in the chest region (Perkins and Ford, 1969; Matz-Rensing and Lowenstine, 2018).

Apocrine scent glands are characterized by the odour of their secretion, which is produced by the action of local bacteria. In humans, the majority of AAC develop in the axilla, followed by the anogenital region, which corresponds to the areas with greatest apocrine gland density (Higgins and Strutton, 1997; Cardoso and Calonje, 2015; Zahid *et al*., 2016; Angelico et al., 2016). The fact that the only case reported to date (Cameron and Conroy, 1976) and the present case, both developed a scent adenocarcinoma in the chest area, is consistent with the high density of apocrine glands in this anatomical region in NHP, particularly NWM (Perkins and Ford, 1969; Matz-Rensing and Lowenstine, 2018).

The neoplasm described in this report had a predominantly tubular arrangement with areas of solid growth, which is the most common pattern in human AAC (Cardoso and Calonje, 2015). In contrast, the case reported by Cameron and Conroy (1976) had a predominantly papillary pattern. Furthermore, it is impossible to discern from this earlier publication if myoepithelial cells were involved in the neoplasm. In humans the participation of myoepithelial cells in AAC is rare but common in dogs and infrequent in cats (Haziroglu *et al*., 2014; Cardoso and Calonje, 2015; Goldschmidt and Goldschmidt, 2017). In the present study, immunolabeling of 34βE12, CK5, CK14 antigens demonstrated that myoepithelial cells were not involved in the neoplasm, which is consistent with a diagnosis of simple apocrine adenocarcinoma. The presence of myoepithelial cells is commonly considered an indicator of better prognosis because they act both as a physical barrier that prevents invasion and as tumour suppressors in a paracrine manner (Sabater Marco, 2012; Suarez-Bonnet *et al*., 2017). The absence of this cell type in our case may suggest malignancy. However, this absence cannot be considered as a definitive criterion of malignancy as some benign proliferative apocrine lesions also lack a myoepithelial compartment (Cserni, 2008).

The cytokeratin immunohistochemical profile of the present case is similar to that reported in AAC of human beings and domestic animals (Collina *et al*., 2004; Santa Cruz, 2007; Miyamoto

166 *et al*., 2009; Cardoso and Calonje, 2015, Prieto, 2019). The cytokeratin profile of apocrine and
167 eccrine glands is similar and includes expression of CK7, CK8 and CAM 5.2. Gross cystic
168 disease fluid protein (GCDFP-15) is claimed to be a reliable marker to differentiate apocrine
169 glands from eccrine glands (Collina *et al*., 2004; Santa Cruz, 2007; Prieto, 2019). GCDFP-15
170 but has not been used in veterinary species and could not be included in this study. While
171 human ACC is frequently immunopositive for CEA, this tumour was negative, most likely due
172 to an absence of cross-reactivity with the antibody used. Human ACC are rarely
173 immunopositive for CK19 which is believed to be a marker of ductal origin (Yamamoto *et al*.,
174 2000). There is variability in the expression of ORα and PR in human sweat-gland tumours
175 with both positive and negative cases reported, although the clinical significance for both
176 scenarios has not been determined (Sabater Marco, 2012; Cardoso and Calonje, 2015). The
177 Ki67 PI of our case was 12% compared with a range of 9.45% to 59.20% in human ACC. AAC
178 with a high (> 10%) PI may be associated with neoplastic infiltration of the overlying epidermis
179 (extramammary Paget's disease), as described by Miyamoto et al. (2009) but evidence of
180 epidermal involvement in the non-ulcerated epidermis was not observed in this case.
181 A principal differential diagnosis for AAC is mammary adenocarcinoma. However, the sex of
182 this animal (male), the anatomical location, histopathological features (cytoplasmic blebbing
183 and decapitation secretion) and immunohistochemical profile support an apocrine origin.
184
185 In human beings and domestic animals, AAC can behave aggressively and metastasise to
186 distant organs including bone (Gross *et al*., 2005; Cardoso and Calonje, 2015; Goldschmidt
187 and Goldschmidt 2017). A routine follow-up of this case revealed that the animal was still
188 alive, with no evidence of clinical disease 24 months after surgery. The complete excision of
189 the neoplasm was therefore deemed to be curative and the absence of neoplastic emboli within
190 lymphatics was a histological criterion of good prognosis.

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Conflict of Interest Statement

The authors declared no potential conflicts of interest with respect to the research or publication of this article.

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Figure Legends

Figure 1. Apocrine (scent) gland adenocarcinoma a wedge-capped capuchin monkey. The thoracic mass is firm and well-demarcated, but adhered to underlying tissue and extensively ulcerated. Bar, 3 cm.

Figure 2. Apocrine (scent) gland adenocarcinoma in a wedge-capped capuchin monkey. Epidermis is extensively ulcerated (arrows). A bilobulated mass compresses adjacent non-neoplastic apocrine acini (arrowheads). HE. Bar, 5 mm. Upper inset: Marked anisokaryosis, anisocytosis, macrokaryosis and frequent mitoses. Haemorrhage in a narrow tubular lumen. HE. Bar, 25 μm. Lower inset: Dysplastic apocrine acini surround the neoplasm and have occasional loss of polarity, hyperchromatic or binucleated nuclei. Moderate neutrophilic infiltrate. HE. Bar, 50μm.

Figure 3. Apocrine (scent) gland adenocarcinoma in a wedge-capped capuchin monkey. Intense cytoplasmic and membranous labelling of CK 8 antigen in neoplastic cells and prominent collagenous spherulosis (asterisks). IHC. Bar, 100 μm. Inset: Absence of CK 8 immunolabeling in overlying epidermis. IHC. Bar, 100 μm.

Figure 4. Apocrine (scent) gland adenocarcinoma in a wedge-capped capuchin monkey. Left: Myoepithelial cells of non-neoplastic apocrine glands are intensely labelled for CK 14 antigen. IHC. Bar, 100 μm. Right: Complete absence of myoepithelial cells within the neoplasm. IHC. Bar, 100 μm.

Supplementary Figure 1. Prominent bands and cords of collagen (asterisks) between neoplastic tubules (collagenous spherulosis). HE. Bar, 100 μm.