

Well-differentiated Squamous Cell Carcinoma in a Captive Clouded Leopard (*Neofelis nebulosa*)

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Abstract

Squamous cell carcinoma (SCC) is a malignant neoplasm of keratinized epithelial cell affecting both domestic and wild animals. A few cases of SCC have been reported in wild felids. A fifteen year old, intact female, clouded leopard showed signs of lameness and swelling of the right hind paw, which upon physical examination was noted to contain an abscess. Surgical biopsy of the site revealed a well-differentiated SCC. Attempts at surgical excision and cryosurgery proved unsuccessful and there was a recurrence of the mass. Therefore, mid-femoral amputation was performed. Macroscopically, the amputated limb revealed multiple dermal, cavitated whitish masses. Microscopically, the neoplastic cell revealed typical features of SCC and immunoreactive to cytokeratin in the cytoplasm of the neoplastic cells. This case report is the first to describe a well-differentiated SCC in a clouded leopard.

Keywords: captive, clouded leopard, paw, immunohistochemistry, squamous cell carcinoma

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Introduction

Squamous cell carcinoma (SCC) is a malignant tumor of keratinized epithelial cell that is commonly reported as affecting a wide range of tissue types throughout the body in domestic animals (Goldschmidt and Hendrick, 2002). SCC is behaviorally locally aggressive and frequently metastasized to regional lymph nodes, while distant metastases are rarely reported (Goldschmidt and Hendrick, 2002). Although SCCs, being a commonly diagnosed neoplasm, have been described in a wide variety of zoo and wild species (Marder et al., 2000; Ewing and Mignucci-Giannoni, 2003; 2005; Goodman et al., 2007), only five cases of SCC have been documented in wild felids. These reports include a Canadian lynx (Gudson et al., 1978), an ocelot (Yanai et al., 2003), a tiger and a jaguar (Owston et al., 2008), and an African lion (Mwase et al., 2013). However, SCCs have not been described in clouded leopards (*Neofelis nebulosa*), which is a medium-size wild felid and was classified as a vulnerable C1+2a(i) version 3.1 on the IUCN Red List of Threatened Species (2013). This case report describes both spontaneous well-differentiated SCC in the right hind paw of a captive clouded leopard and its treatment.

Materials and Methods

A fifteen year old, intact female clouded leopard (*Neofelis nebulosa*) was transferred from an open zoo in the Eastern part of Thailand to Dusit Zoo in Bangkok 3 years prior to showing signs of lameness in its right hind-limb. Physical examination revealed focal swelling of the plantar site of the right hind paw measuring 1 cm in diameter with deep ulceration of the epidermis and extrusion of purulent exudate. *Enterobacter agglomerans* and *Kleibella pneumoniae* were isolated from the wound. The leopard was treated with wound dressing and antibiotic via intramuscular injection, which resulted in only partial resolution. The mass was subsequently biopsied and examined histopathologically. The diagnosis was of a well-differentiated SCC. Surgical excision was performed accompanied by cryosurgery in an attempt to completely excise the neoplasm and to cauterize the wound due to the lack of available tissue on the paw for suturing. Unfortunately, the mass locally recurred within a month of post-operation. A mid right femoral amputation was performed and all tissues were fixed in 10% neutral buffered formalin. The fixed mass of the right hind paw was embedded in paraffin wax and cut at 4 µm thickness. Sections were stained with Hematoxylin and Eosin (H&E). Immunohistochemistry using the envision system was also performed using anti-cytokerin (AE1/AE3) antibody as a primary antibody, 3,3'-diaminobenzidine (DAB, DAKO) as chromogen and Mayer's hematoxylin as counterstain.

Results and Discussion

Macroscopically, the right hind-limb revealed swelling of the paw with superficial

ulceration. On incision, the lesion consisted of multiple irregular, cavitated whitish masses measuring 4 cm (Fig 1).

Microscopically, unencapsulated mass was composed of a dense cell of epithelial neoplasm. The cord-like structure and island of epithelial neoplasm were infiltrated from the epidermis into the deep dermis (Fig 2). Neoplastic cells were polygonal with distinct cellular borders widely separated by a densely packed collagenous stroma. The neoplastic cells had moderate to abundant eosinophilic cytoplasm, and large round nuclei with single prominent nucleolus. Laminated keratin material (keratin pearl) was frequently noted in the center of the neoplastic islands. A high mitotic rate was noted (3-4 cells/HPF). The neoplastic islands also infiltrated into the digital tendon (Fig 3) of the paw, indicating that the neoplastic cell had undergone local invasion into adjacent tissues, which is frequently described in SCCs (Goldschmidt and Hendrick, 2002; Yanai et al., 2003). The cytoplasm of the neoplastic cells was strongly and diffusely immunoreactive to cytokeratin (AE1/AE3) (Fig 4). Taken together, a spontaneous well-differentiated SCC was diagnosed based on histopathological findings and strong immunoreaction to cytokeratin in the cytoplasm of neoplastic cells suggesting that the neoplastic cells were of epithelial origin as previously described (Ewing and Mignucci-Giannoni, 2003; Yanai et al., 2003). Although SCC had been reported in five species of wild felid (Gudson et al., 1978 ; Yanai et al., 2003; Owston et al., 2008; Mwase et al., 2013), there are no reports in clouded leopards. This case report is the first to describe a spontaneous well-differentiated SCC in a clouded leopard. A spontaneous SCC was suggested in the present case based on the age of the leopard. The average lifespan of the captive clouded leopards is 13-15 years (Holmes, 2009). A longer lifespan due to good management of captivity is a potential risk of SCC development (Goodman et al., 2007; Owston et al., 2008). In addition, many etiologies included chemical carcinogen, prolonged UV light exposure and papillomavirus infection as potential causes of SCC in both domestic and wild animals (Goldschmidt and Hendrick, 2002; Yanai et al., 2003). Although this leopard was kept in the rural zoo for 12 years prior to being moved to the urban zoo, it is possible that exposure to environmental carcinogen promoted the formation of the neoplasm in this instance (Yanai et al., 2003). Incomplete removal of the mass likely resulted from both the lack of sufficient tissue for wide margins and the neoplastic cells already undergoing local invasion, since it was subsequently observed in the digital tendon (Goldschmidt and Hendrick, 2002). The leopard revealed a good quality of life including eating, walking, and other appearances since the mid-femoral amputation had been performed, indicating that this modality is a useful treatment option for animals suffering from digital SCC as previously described in a white rhinoceros (Goodman et al., 2007). Unfortunately, the leopard died within one year and one month later due to chronic renal failure. However, no evidence of the neoplastic cells was observed in regional lymph node and other organs.



Figure 1 Cross-section of the right hind paw showed irregular, cavitated whitish masses (open star) and ulceration of the skin (arrow; inset).

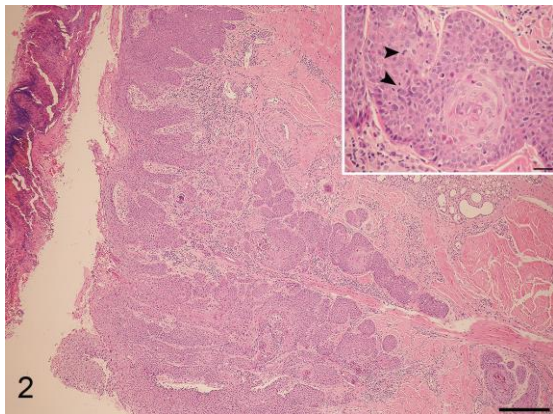


Figure 2 Cluster of SCC invaded the deep dermis with superficial necrosis and acanthosis of the epidermis (H&E stain, bar = 200 µm). Inset: Neoplastic cells are polygonal with distinct cellular borders, are moderate to abundant eosinophilic cytoplasm, have large round nuclei containing a single prominent nucleolus and have high mitotic activity (arrowheads) (H&E stain, bar = 20 µm).

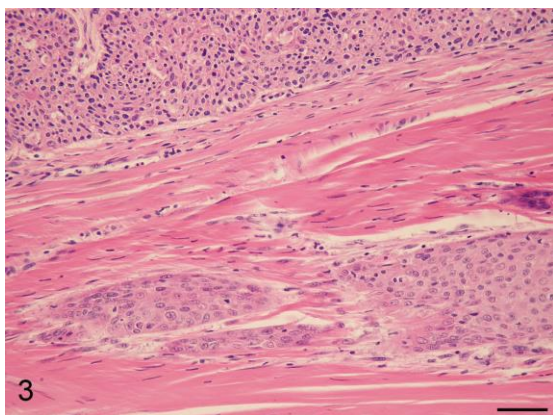


Figure 3 Invasion of SCC observed within the digital tendon (H&E stain, bar = 40 µm).

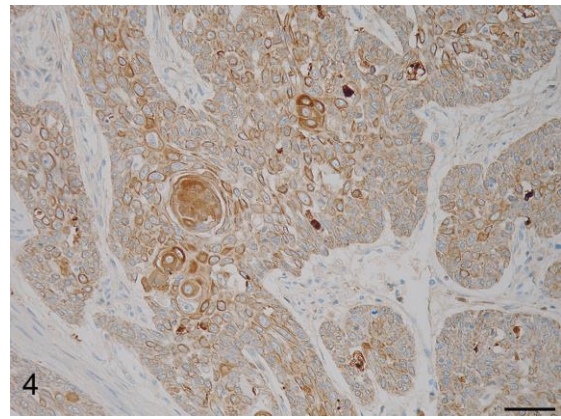


Figure 4 Neoplastic cells demonstrated strong and diffuse immunoreaction in the cytoplasm (IHC Envision system, DAB, Bar = 40 µm).

Interestingly, a swollen wound with discharging sinus tract was described as an important feature of SCC in human (Pahwa et al., 2011) and lion (Mwase et al., 2013), comparable with the signs of swelling, lameness, and exudation of the right hind paw observed in the clouded leopard.

This report is the first to describe a spontaneous well-differentiated SCC in a clouded leopard that was successfully treated by mid-femoral amputation.

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บทคัดย่อ

มะเร็งผิวหนังชนิดสความัสเซลล์คาร์ซิโนมาในเสีอลายเมฆที่เลี้ยงในกรง

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มะเร็งผิวหนังชนิดสความัสเซลล์คาร์ซิโนมาเป็นมะเร็งที่มีรายงานทั้งในสัตว์เลี้ยงและสัตว์ป่า ในสัตว์ป่าตระกูลแมวมีรายงานการตรวจพบมะเร็งผิวหนังชนิดสความัสเซลล์น้อย เสีอลายเมฆ เพศเมีย อายุ 15 ปี มีปัญหาเดินลำบากร่วมกับการบวมและสิ่งคัดหลั่งที่เป็นหนองที่อุ้งเท้าหลังข้างขวา การตรวจวินิจฉัยชิ้นเนื้อพบมะเร็งผิวหนังชนิดสความัสเซลล์คาร์ซิโนมา จึงทำการผ่าตัดเอาก้อนเนื้อออกร่วมกับการผ่าตัดด้วยความเย็น แต่กลับมาเป็นซ้ำอีก จึงได้รับการผ่าตัดเอาขาหลังออกและสิ่งชิ้นเนื้อตรวจทางพยาธิวิทยา การตรวจทางพยาธิวิทยา พบก้อนเนื้อสีขาวจำนวนหลายก้อนในขาหลังร่วมกับการเกิดแผลหลุมและโพรงเนื้อตาย จากการตรวจทางจุลพยาธิวิทยาสรุปได้ว่า เกิดมะเร็งผิวหนังชนิดสความัสเซลล์คาร์ซิโนมา ซึ่งให้ผลบวกต่อการตรวจไซโตเครตินด้วยอิมมูโนฮิสโตเคมี การศึกษาครั้งนี้รายงานการเกิดมะเร็งผิวหนังชนิดสความัสเซลล์คาร์ซิโนมาในเสีอลายเมฆเป็นครั้งแรก

คำสำคัญ: ขังกรง เสีอลายเมฆ ขาหลัง อิมมูโนฮิสโตเคมี มะเร็งผิวหนังชนิดสความัสเซลล์คาร์ซิโนมา

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