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NOTE

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# Squamous cell carcinoma in a digit of the hind limb with systemic metastasis in a 17-year-old female koala

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**ABSTRACT.** We encountered a case of cutaneous squamous cell carcinoma (SCC) in a 17-year-old female koala at a zoo. A fragile, papillary, elevated mass was found on the third digit of the right hind limb. SCC was identified histopathologically: squamous cell-like polygonal tumor cells showed a nest-like growth pattern with epidermal down growth, central keratinization and necrotic foci, and invaded dermal connective tissues. Metastatic lesions were observed in various organs, including the lung and axillary lymph node: in the lung, multiple metastatic foci similar to the primary lesion, and in the axillary lymph node, individual polygonal tumor cells infiltrated the sinusoids. Immunohistochemistry revealed that the tumor cells were positive for proliferating cell nuclear antigen, which exhibited 32–33% of labeling indices in the tumor cells. To our knowledge, this is the first report of a case of SCC in a digit of a koala.

**KEY WORDS:** digit, koala, squamous cell carcinoma

Neoplastic lesions commonly found in wild and captive koalas frequently show lymphoma and leukemia because most koalas are infected with the koala retrovirus (KoRV) [6]. The incidence of other neoplastic lesions, including squamous cell carcinoma (SCC), is also relatively high in koalas. We encountered a case of SCC in a digit of the hind limb with systemic metastasis in a 17-year-old female koala at a zoo.

A 17-year-old female koala that was bred at Hirakawa Zoological Park (Kagoshima, Japan) showed slight swelling of the tip of the right third digit of the hind limb followed by papillary growth of the tumor mass. Palpation revealed swelling of several parts of the body: right axillary lymph node (3 cm diameter), right axillary subcutaneous mass (1 cm diameter), and masses in mammary glands (3 × 2 × 2 cm on the right side and 2 × 1 × 1 cm on the left side). The animal had been receiving veterinary palliative care, and could stay in the tree forks, handle branches and consume leaf (eucalyptus) until the day before it died. According to the code of Practice for Injured, Sick and Orphaned Koalas [13], euthanasia was not performed in the case.

Macroscopically, we observed an ulcerated mass (5 × 3 × 2 cm diameter) in the right hind leg, swelling of the right axillary lymph node, a right axillary subcutaneous mass, yellowish white nodules in the dark brown liver, and ascites. Tissue samples were collected from the heart, lung, liver, spleen, kidney, adrenal gland, pancreas, gallbladder, ovary, uterus, urinary bladder, stomach, small and large intestines, cervical and right axillary lymph nodes, the right axillary subcutaneous mass, mammary glands, and femoral bone marrow. Tissue samples were fixed in 10% neutral buffered formalin and then embedded in paraffin wax, sectioned at 3–5 µm, and stained with hematoxylin and eosin. Serial sections were also immunohistochemically stained using the streptavidin-biotin complex method. The primary antibodies used are listed in Table 1. For negative controls, the primary antibodies were replaced with non-immunized sera; for positive controls, the positive reactions were confirmed in normal epidermis and connective tissues for cytokeratin AE1/AE3, cytokeratin 5 (CK5), α-smooth muscle actin (α-SMA), β-catenin and proliferating cell nuclear antigen (PCNA). Immunostaining results (labeling index, LI) of PCNA were quantified by the number of tumor cell nuclei as a percentage of 1,000 nuclei counted in the digital mass, lung and axillary lymph node [3].

Histologically, the tumor mass of the right third digit of the hind limb grew from normal epidermis with epidermal down growth and expanded widely to the dermis and subcutaneous tissue. The tumor consisted of variable-sized nests or cords of polygonal squamous cells, occasionally forming central keratinization (Fig. 1A). The tumor cells frequently infiltrated the dermis and

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**Table 1.** Antibodies used in immunohistochemistry

| Antigen | Host species | Clonality | Dilution | Antigen retrieval | Manufacture |
|---|---|---|---|---|---|
| Cytokeratin AE1/AE3 | Mouse | Monoclonal | Undiluted | Autoclaved[a] | Dako (Glostrup, Denmark) |
| CK5 | Rabbit | Monoclonal | 1:400 | Microwaved[b] | Abcam Inc. (Tokyo, Japan) |
| α-SMA | Mouse | Monoclonal | 1:500 | Microwaved[b] | Dako |
| β-catenin | Rabbit | Polyclonal | 1:250 | Microwaved[c] | Dako |
| PCNA | Rabbit | Polyclonal | 1:200 | n.a. | Dako |

α-SMA, α-smooth muscle actin; CK5, cytokeratin 5; n.a., not applicable; PCNA, proliferating cell nuclear antigen. [a] At 121°C for 10 min in 10 mM citrate buffer (pH 6.0), [b] at 90°C for 10 min in Dako target retrieval solution (pH 9.0), [c] at 90°C for 10 min in 10 mM citrate buffer (pH 6.0).

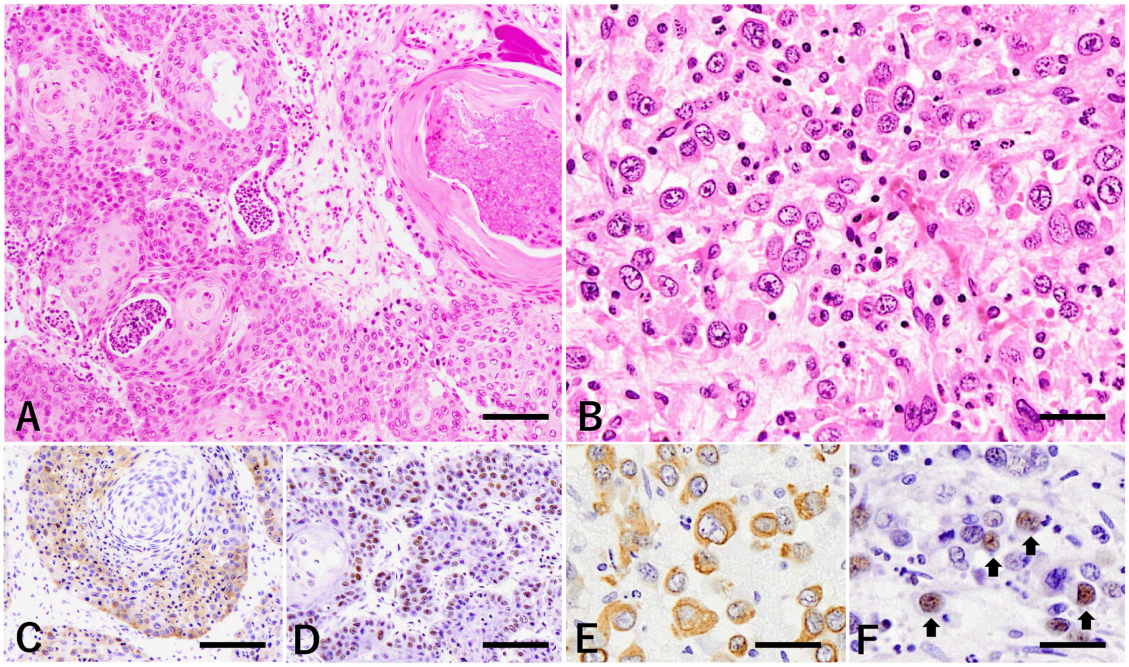


**Fig. 1.** Histopathological and immunohistochemical findings of squamous cell carcinoma in the digit and axillary lymph node of a koala. (A) Squamous tumor cells in a nest or cord-like growth pattern, with central keratinization in the digital mass. (B) Individual tumor cells in the axillary lymph node. (C–F) The tumor cells are positive for CK5 (C, E) in the cytoplasm, and proliferating cell nuclear antigen (PCNA) (D, F) in the nuclei of tumor cells (F: arrows, PCNA-positive nuclei) in the digital mass (C, D) and axillary lymph node (E, F). Bar=50 μm (A, C, D) and 12.5 μm (B, E, F).

subcutaneous tissue, forming isolated small tumor foci or showing invasive growth of single cells in interstitial tissues. The tumor cells had round to oval or vesicular nuclei with one or two prominent nucleoli and abundant eosinophilic cytoplasm. Necrotic foci with surrounding granulation tissues accompanied by mononuclear cell infiltration were frequently observed.

Metastatic tumor cells were observed in multiple tissues. In the lung, multiple metastatic foci replaced the normal alveobronchiolar structure; the polygonal squamous cells resembling those of primary lesions grew in a similar way and sometimes showed central keratinization. Multilayered thrombi with cell debris were found in pulmonary arteries surrounded by granulomatous fibrous tissues. Tumor cells also infiltrated the pleural connective tissues, heart, liver, spleen, kidney, urinary bladder, adrenal gland, and subcutaneous tissues in the right axillary subcutaneous mass and mammary glands. In the lymph nodes, tumor cell invasion was prominent with congestion and sinusoidal infiltration of inflammatory cells (Fig. 1B), and the lymph node structures were mostly replaced by tumor cells. Tumor cells were observed in the afferent and efferent lymph ducts. In the other tissues, no significant pathological changes were observed.

Immunohistochemical analysis showed that the tumor cells in the primary and metastatic lesions were strongly positive for cytokeratin AE1/AE3 and CK5 (Fig. 1C and 1E) in the cytoplasm, and negative for α-SMA. Positive reactions for PCNA (Fig. 1D and 1F) in the nuclei were observed. Nuclear labeling of PCNA was consistent in the primary right hind limb (32.2% of LI) and metastatic masses in the lung (33.1% of LI) and right axillary lymph nodes (32.2% of LI). β-catenin was detected in the cytoplasm, but not in the nuclei.

In dogs, the digits are involved in malignant and benign tumors and inflammatory conditions [15]. SCC is the most common

type of malignant tumor diagnosed in digits of dogs [8, 15]. To our knowledge, this is the first report of SCC in a digit of a koala, with a higher proliferation activity of tumor cells as shown by PCNA LI than that (2.1–4.8%) in SCC of canine skin [9]. In dogs, the forelimb has been reported to be affected twice as much as the hind limb [4], and multiple digits can be affected (7.9%) [15]. In the present case, SCC occurred in the third digit of the right hind limb and did not affect other digits. The present study provides evidence regarding metastasis of digital SCC in a koala, which is in agreement with digital SCC having a greater metastatic potential than SCC found on the body in dogs [15]. Nail bed SCC was excluded by clinical and macroscopic observations, although it is known to be involved in canine digital SCC [4].

Papillomaviruses are small, non-enveloped, icosahedral viruses that infect the stratified squamous epithelium of many mammals, including humans, as well as some avian and reptilian species [11, 14]. The known non-human papillomavirus types are mostly mammalian types, but also include three avian and three reptilian types, and skin papillomaviruses can readily be detected in healthy skin from many different animal species [1]. These animal papillomavirus types were sufficiently genetically related to their human counterparts to be identifiable by a human skin papillomavirus primer set (FAP59 and FAP64) [5]. Furthermore, in Australia, three newly identified papillomavirus types in koala, such as KoAA1 (analysis of the 440-bp cloned fragment from FAP polymerase chain reaction showed the closest similarity to HPV 1a), KoAA2, and KoAA3 (closest similarity to KoAA1) have been detected using primer sets (FAP59 and FAP64, and KoPVFAP59 and KoPVFAP64 designed for koala papillomavirus type) [2]. In particular, digital SCC in humans is frequently associated with high-risk, oncogenic human papillomavirus subtypes and recurrence, and aggressive treatment is recommended [7]. We did not detect the genes of KoAA1 and other papillomavirus using DNA isolation from paraffin blocks using a NucleoSpin® DNA FFPE XS (MACHEREY-NAGEL, Düren, Germany) and the primer sets (FAP59 and FAP64, and KoPVFAP59 and KoPVFAP64) [2]. The results were consistent with the histopathological findings that we did not observe papillomavirus-related cytopathology, epidermal cells with extended grayish blue cytoplasm and koilocytes with dark shrunken nuclei surrounded by a clear cytoplasm [10, 12].

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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