Non-metastatic squamous cell carcinoma in two Hermann’s tortoises (Testudo hermanni)

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Abstract

Squamous cell carcinomas (SCC) are malignant tumors of the epidermal cells with varying degrees of keratinocyte differentiation. They are common tumors in mammalian and avian species but there are, however, only two description of SCC in tortoises. In this report we describe two cases of non-metastatic squamous cell carcinomas of the carapax and the plastron in Hermann’s tortoises with evidence of humoral hypercalcemia of malignancy (HHM) in one case. HHM is thought to be associated with SCC in mammals due to de novo secretion of parathyroid hormone-related protein (PTHrP) by the tumor cells or tumor induced osteolysis but has not been described in reptiles so far.

Introduction

Due to an increased life expectancy of non-domestic animals in captive care tumors are recorded more often, when compared with free ranging animals.1 In reptiles, neoplastic diseases are observed in all three major orders with snakes having the highest prevalence, followed by lizards, chelonians and crocodilians.2 The skin is one of the most common tumor sites in reptiles with frequent descriptions of fibrosarcomas, papillomas and pigment cell tumors.1,4 Squamous cell carcinomas (SCC) are most frequently observed in snakes and lizards.5,6 Within the order of testudines several cases of SCC have been described in turtles.2,7 However, in the family testudinidae (tortoises) only two cases of SCC have been reported so far, in a Brazilian giant tortoise (Chelonoidis denticulata, formerly Geochelone denticulata)12 and in a Hermann’s tortoise.13 The skin is the most common site to develop SCC in chelonians but oral,12 and coelomic8 SCC can also be present. Metastatic spread of SCC in reptiles is rare and has been reported in only three cases of turtles.7,9

Humoral hypercalcemia of malignancy (HHM) is a paraneoplastic syndrome commonly associated with several mammalian tumors but to the authors knowledge it has not been described in reptiles. HHM is caused by de novo secretion of parathyroid hormone-related protein (PTHrP) by the tumor cells or thought to be associated with tumor induced osteolysis.14

The present case report describes two cases of SCC in Hermann’s tortoises without metastatic spread but in one case with clinical and pathologic lesions similar to HHM in mammals.

Case Report

Anamnesis and clinical findings

Animal #1, a 28 year old male Hermann’s tortoise (Testudo hermanni), was presented due to a profound swelling at the tail base for several weeks. The single kept tortoise lived on a balcony during summer and was running free in the apartment during winter. Hibernation was not performed. Salad and chicory were the main components of the tortoise’s nutrition. Neither a heating place or ultraviolet light nor vitamin or mineral supplements were provided for the animal. The clinical examination revealed a 1.13 kg apathetic and cachectic. Animal #1 had a solid and grey palpable tumor on the left, caudal quadrant of the plastron over a period of 6 to 7 months. The ulcer was treated locally with antibiotics. The animal died spontaneously and was submitted for necropsy.

Histopathology

On post mortem examination both tortoises were cachectic. Animal #1 had a solid and grey to yellow mass with a size of 7.0 x 5.0 x 3.0 cm, expanding from the right side of the tail to the caudal right coelom involving the caudal inner side of the carapax (Figure 3). The urinary sac was distended by approximately 40 mL clear, yellow, slightly gelling urine. The digestive tract was empty. The parathyroids were unremarkable.

Animal #2 had a large perforating ulcer, measuring 8.0 x 5.0 cm in the left, caudal quadrant of the plastron with irregular borders (Figure 4). On cut section, the ulcer was encircled by a dense, irregular, grayish mass with central necrosis and mild to severe reactive new bone formation. Besides, both knee joints had mild, multifocal degenerative joint disease with loss of joint cartilage. The faeces were formed and contained numerous nematodes consistent with Oxyuris spp.

Histologically, the mass of animal #1 was identified as a moderately differentiated SCC. The tortoise developed ulceration on the left, caudal quadrant of the plastron over a period of 6 to 7 months. The ulcer was treated locally with antibiotics. The animal died spontaneously and was submitted for necropsy.
replacing the lamellar bone of the carapax bone plates (Figure 5). Adjacent to this area a severe, subacute, multifocal, heterophilic osteomyelitis was evident. Multifocal within the tumor keratin pearls were evident and individual cells often showed irregular keratinization (Figure 6). The extensive coagulative necrosis was mostly admixed with moderate numbers of heterophils and showed multifocal sites of mineralization and occasionally evidence of basophilic coccoid bacteria. There was infiltration of the adjacent tissue by tumor cells but neither blood nor lymphatic vessels contained tumor cells. Neither PAS-positive fungi nor acid fast bacteria could be detected. Furthermore a mild, subacute, multifocal, interstitial nephritis with mild tubular mineralization and dilatation was identified. A moderate, subacute, multifocal, heterophilic mineralization was evident. Within the stomach moderate, multifocal mineralization was evident. Brain, lung, heart, liver, digestive tract and skeletal muscle were unremarkable.

The histological examination of the ulcerated mass of animal #2 was also identified as a moderately differentiated, infiltrative SCC without invasion of surrounding vessels. The polygonal to fusiform cells showed mostly distinct cell borders and moderate amounts of eosinophilic, homogenous cytoplasm. The mitotic rate was low with less than one mitotic figure per high power field. Individual cells revealed irregular keratinization but only few keratin pearls were visible. There were multifocal small areas of coagulative necrosis admixed with few lymphocytes and plasma cells. Within the mass multifocally basophilic, coccoid bacteria and occasionally mineralization were evident. Around the nests of tumor cells there was a desmoplastic reaction in the surrounding tissue. Few PAS-positive fungal hyphae were present besides the areas of necrosis. Acid fast bacteria were not detectable. Metastatic tumor cells were not present in lung, heart, liver, digestive tract or skeletal muscle.

In summary, macroscopic and histologic findings lead to the diagnosis of a non-metastatic squamous cell carcinoma in both cases.

### Discussion

Here, we describe two cases of non-metastatic squamous cell carcinoma in Hermann’s tortoises. Squamous cell carcinomas (SCC) are rarely reported to occur in the order testudines. Two reports identified this neoplasm in testudinidae (tortoises): A Brazilian giant tortoise (Geochelone denticulata), and a Hermann’s tortoise (Testudo hermanni) whereas eight cases of squamous cell carcinomas were described in turtle species others than testudinidae: An Indian black turtle (Melanochelys trijuga), an European pond turtle (Emys orbicularis), an Eastern box turtle (Terrapene carolina), a Red-eared slider (Trachemys scripta elegans), a Common snake-necked turtle (Chelodina longicollis), a Spiny soft shell turtle (Apalone spinifera spinifera) and two Loggerhead sea turtles (Caretta caretta). The neoplasm appeared at different locations, mostly the skin but also included the oral cavity and the coelom. Metastasis has not been reported in tortoises and was not present in the two cases reported here. In a pond turtle with SCC of the intermandibular space metastases were found in the liver. Furthermore, two loggerhead sea turtles with multiple SCCs showed metastases in several tissues. Other reports of SCC in reptile species did not mention metastasis. This is in contrast to the situation in mammals where metastasis is a common phenomenon in patients with oral but also with cutaneous SCC.

Animal #1 had a hypercalcemia of unknown origin. The main causes of hypercalcemia in animals are increased calcium mobilization from bone, increased calcium absorption from the intestine, decreased urinary excretion of calcium due to renal failure or increased protein-bound calcium or hypercalcemia of malignancy (HHM). Increased protein bound calcium could be excluded in the present case because free calcium was measured. Primary hyperparathyroidism was not likely to be the cause of the increased free calcium since there were no clinical or macroscopic observable lesions in the parathyroids. Secondary renal hyperparathyroidism was excluded since the interstitial nephritis in this case was only mild and subacute. Additionally, phosphorus concentrations in the blood were within reference ranges. The animal neither received any vitamins, nor had contact to plants or substances known to have vitamin D activity, therefore a hypervitaminosis D as the cause of hypercalcemia was unlikely. The tortoise did not have any granulomatous inflammation which may lead to Vitamin D synthesis by macrophages.

Exclusion of this common causes of hypercalcemia in this case leads to the potential diagnosis of humoral HHM due to an increased synthesis of PTHrP by the tumor cells or tumor-induced osteolysis of the carapax. Unfortunately, methods to reliably measure
the blood and tissue levels of PTHrP in reptile species are not available. In previous reports, hypercalcemia has not been described in reptiles with SCC but can be observed in humans cats and dogs with HHM.19-21 The basis of the HHM in some of these patients could be associated with PTHrP synthesis by tumor cells,22,23 whereas in others PTHrP synthesis was not identified.24

In summary, SCC is a rare tumor in tortoises, although it is described in a few cases. Both tumors appeared at sites with little exposure to UV light when compared to other regions of the integument, indicating that radiation may not be associated with SCC induction in this species. Both animals were older than 20 years, suggesting that SCC genesis may be associated with age. In addition, at least in one of the animals a hypercalcemia of malignancy was diagnosed, although its exact molecular mechanism remains unclear.

References