

LOW-GRADE FIBROSARCOMAS IN GREEN TURTLES (*CHELONIA MYDAS*) IN THE HAWAIIAN ISLANDS

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ABSTRACT

Fibropapillomatosis in green turtles (*Chelonia mydas*) has been recognized as one of the most important mortality factors in stranded turtles in the Hawaiian Islands. Several viruses including herpesviruses, a retrovirus, and a papilloma-like virus have been associated with the tumors but the causative agent has not been characterized. Histologically, some tumors of the nasopharynx, mouth and temporomandibular tissues appear to have an aggressive, invasive behavior. These masses are well demarcated from adjacent tissues but demonstrate infiltration of surrounding stroma and bone lysis. Although there is no evidence of vascular invasion or high mitotic activity, these tumors have been classified as low-grade fibrosarcomas.

INTRODUCTION

The green turtle (*Chelonia mydas*) is protected under the U.S. Endangered Species Act and the Wildlife Laws of the State of Hawaii. Fibropapillomatosis (FP) is a disease of marine turtles characterized by multiple cutaneous masses ranging from 0.1 to more than 30 cm in diameter that has primarily affected green turtles. The disease has a worldwide distribution and has been observed in all major oceans and in all species of marine turtles that are considered endangered of extinction. Where present, prevalence of the disease varies among locations, ranging from as low as 1% to as high as 90%. Although several viruses have been identified associated with the tumors, including herpesviruses, a retrovirus and a papilloma-like virus, the primary etiological agent remains to be isolated and identified. Concurrent infections of FP and cardiovascular trematodiasis have been recognized as important mortality factors of Hawaiian green turtles considerably reducing the survival of the species.

The neoplastic processes previously observed by Aguirre and Spraker (1995) and more recently during gross and histopathologic examination of 14 turtles collected in the Hawaiian Islands with FP suggested a synergistic effect of cardiovascular trematodes and the primary agent of FP. Tumors in the internal organs of some turtles were characteristic of fibropapillomas, fibromas, myxomas, and fibrosarcomas. This study suggested that, when occurring together, spirorchidiasis and fibropapillomatosis represent a debilitating and fatal syndrome of Hawaiian green turtles.

We describe the gross and histopathology of temporomandibular

tumors found in green turtles from the Hawaiian Islands, and present histopathological evidence suggestive of the presence of low-grade fibrosarcomas.

METHODS

Stranded green turtles with FP have been recovered for detailed gross and histopathologic examination. Also, live turtles have been captured unharmed using SCUBA in Kaneohe Bay for sampling since 1991. All of these turtles were strong and appeared to be relatively healthy and not emaciated. All turtles were released safely back into the ocean after tumors were biopsied. All stranded turtles were transported to the NMFS Honolulu Laboratory for clinical evaluation, euthanasia, and necropsy. Predilection was given to the freshest specimens with FP in the oropharynx. Turtles were humanely euthanized with a lethal intraperitoneal injection of Buthanasia-D Special Solution (Schering-Plough Animal Health, Kenilworth, NJ, USA). Detailed necropsies were performed following the protocol described by Wolke and George (1981). After external examination of skin, head, and appendages, the plastron and muscle masses of the pectoral girdle were removed and viscera examined in situ. Special examination of the buccal cavity, nares, tongue, soft and hard palates, pharynx, larynx, glottis and eyes was performed and the tumors were recorded. Standard techniques were followed for the histopathologic evaluation of specimens. Specimens of normal and affected tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned 6-m thick and stained with hematoxylin and eosin (H&E). Selected tissues were also stained with Masson's Trichrome.

RESULTS

For the purposes of this study, a total of 54 masses 15 mm or less in diameter were surgically removed from 30 turtles. Tumors were small, white to gray, smooth to verruciform, raised masses on the integument and eyelids. Necropsies of 26 turtles were considered in the study based on freshness of the carcasses and location of tumors.

Five of the stranded turtles with large white multilobulated masses of the temporomandibular area were examined histologically. These tumors appeared to have taken their origin from the angle of the mandible/maxilla. Two of these turtles also had tumors of the glottis. These tumors grew both outward and formed multilobulated masses around the angle of the mandible and inward and occupied the orbit, invaded periorbital salt glands and destroyed bone of the

orbit and hard palate. These retrobulbar masses caused mild protrusion of the globe. These tumors were composed of sheets and thin to broad interweaving bundles of fibroblastic tissue. Neoplastic cells had plump oval nuclei surrounded by an extensive amount of eosinophilic fibrillar cytoplasm. The degree of cellularity of these tumors was considered to be moderate. Mitotic figures were extremely rare. Sections did show invasion of bone. In most areas epidermis covering the tumor had undergone necrosis, but in other areas there was a layer of loose relatively normal subcutaneous tissue separating the tumor from the acanthotic epidermis. Pseudoepithelomatous hyperplasia was not a feature of the epidermis. These tumors were diagnosed as low grade, well-differentiated fibrosarcomas.

DISCUSSION

The tumors observed in Hawaiian green turtles occurring primarily in juvenile turtles do suggest at least infectious agents may play a role in the etiology. Elucidation of the etiological agent or agents was not the focus of the study, but the histological features are similar to the fibropapillomas described in domestic animals.

A histological study of the external and internal tumors of these turtles do show several differences. The fibropapillomas of the glottis were histologically similar to the cutaneous and ocular tumors. These glottal tumors were usually found in turtles that had cutaneous tumors. These tumors appeared to be benign, however caused great distress to the turtles due to their location. These glottal tumors would cause severe difficulty in eating and breathing. The tumors of the temporomandibular area were of fibroblastic origin as the other tumors, but did show marked differences. First, the degree of cellularity was slightly higher and the neoplastic cellular nuclei were large. Bony invasion is observed. Tumors similar to these have been described in large breeds of dogs that histologically appear to be benign fibromas of the maxilla or mandible, but biologically act as malignant tumors. These tumors invade bone and occasionally metastasize to lung and regional lymph nodes. (P.A. Ciekot, B.E. Powers, S.J. Withrow, R.C. Straw, G.K. Ogilvie, S.M. LaRue. 1994. Histologically low-grade, yet biologically high-grade, fibrosarcomas of the mandible and maxilla in dogs: 25 cases, 1982-1991). These temporomandibular tumors were diagnosed as low-grade, well

differentiated fibrosarcomas. Cellular features used for this diagnosis included morphology of individual neoplastic cells, degree of cellularity and invasion and destruction of surrounding bone.

Metastasis or malignancy of FP has not been demonstrated; however, some tumors have shown invasion of bone tissue and some internal organs. Adenomatous changes of an ocular tumor of a green turtle suggesting malignancy were described during the late 1930's. Fibromas in lungs, kidneys, heart, liver, and gastrointestinal tract have been reported in green turtles with FP in Florida and Hawaii. These masses are well demarcated from adjacent tissues but some show infiltration of surrounding stroma.

The association of parasites with tumor formation in animals has been documented in other species. *Spirocerca lupi*, a nematode of dogs and wild carnivores in tropical and subtropical areas, has been associated with the induction of tumors in the esophagus and stomach. Pathologic changes include reactive granulomas that develop around the parasite (esophageal nodules); the fibroblasts in the inflammatory lesions may be metaplastic and appear transitional between a granuloma and a sarcoma; and definite neoplastic transformation to fibrosarcoma and osteosarcoma may occur. Other lesions of *S. lupi* include arterial stenosis, fibrosis, and granulomas; and aortic aneurysms. The exact mechanism of tumor induction by the above endoparasites in these animal species is not well understood. It has been suggested that the secretion of a chemical carcinogen by the parasite may induce neoplasia.

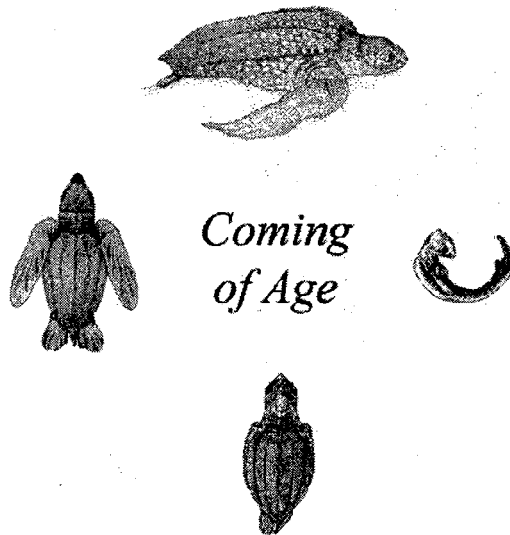
Although there is no evidence of vascular invasion or high mitotic activity, further research is necessary to demonstrate whether the visceral lesions are the result of metastasized external papillomas, or indeed are multiple independent processes. In other species, the precancer to cancer sequence in the progression of canine oral papilloma to carcinoma has been documented. Currently our project is trying to establish the biological behavior and molecular characterization of these tumors.

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