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Fibropapillomatosis in Marine Turtles

ANNIE PAGE-KARJIAN

Fibropapillomatosis (FP) in marine turtles is a debilitating, infectious disease characterized by single or multiple tumors that may develop anywhere on an afflicted turtle's body (Fig. 57.1). FP mainly affects green turtles *(Chelonia mydas)* but has been reported in all marine turtle species.[1–7] There is evidence that FP does not negatively impact green turtle population recovery, survival probability, or somatic growth; however, FP disease may have severe negative effects on the health of an individually afflicted turtle.[8–11] FP tumors are histologically benign; however—depending on their location, size, and degree of invasiveness—they can be fatal in some cases.[1] For example, turtles with periocular or corneal tumors (Fig. 57.2) may have difficulty acquiring food and/or avoiding boats; turtles with oropharyngeal masses may have difficulty feeding and/or breathing; turtles with flipper tumors may have reduced swimming ability and/or be more likely to become entangled in fishing gear; and turtles with internal tumors may experience organ dysfunction and/or physiologic imbalances.[12,13] In free-ranging marine turtles, FP is most frequently observed in juveniles, and the presence of FP-afflicted turtles has been associated with shallow/inshore waters, especially habitats affected by anthropogenic impacts such as agricultural, urban, and industrial development.[1,14–17] FP is a major concern for caretakers of captive or rehabilitating turtles because extensive quarantine measures are necessary for marine turtles with FP, and prognoses for turtles with severe FP are complicated by poor nutritional condition, poor general health on admission, and secondary or opportunistic infections.[18–21]

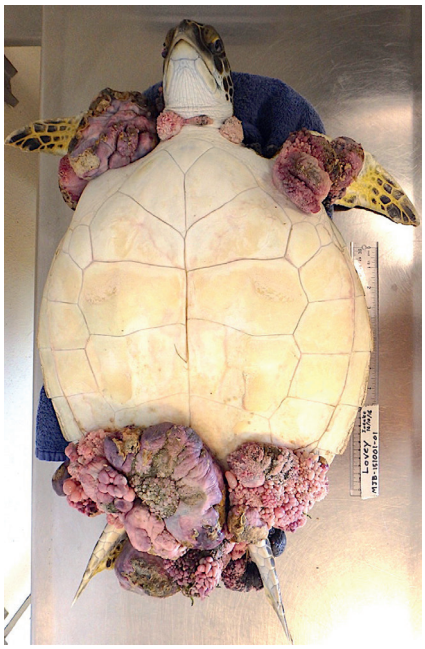
Etiology

Most evidence points to a herpesviral etiology for FP, with the majority of molecular data suggesting that chelonid herpesvirus 5 (ChHV5) is the main causative agent of FP. A series of transmission experiments demonstrated three of the four Koch's postulates, and in vitro replication of the virus was demonstrated when de novo formation of ChHV5-positive intranuclear inclusions were observed in three-dimensional cultures of green turtle skin cells.[1,21–23] A consistently strong statistical association of ChHV5 with FP tumors has been confirmed by many subsequent studies using molecular technologies such as polymerase chain reaction (PCR) and in situ hybridization.[25,26] The virus has been visualized in tumors via transmission electron microscopy (TEM), and immunohistochemistry and reverse transcriptase PCR have further demonstrated that ChHV5 is transcriptionally active in epithelial cells of FP tumors.[21,26–31] FP is a complex disease wherein multiple factors likely play a role in tumor development and progression, including ChHV5 infection as well as environmental, microbial, and/or immune-related cofactors (see also Chapter 39).

Epidemiology

FP disease occurs worldwide but is mainly reported in warmer waters in and around the tropics.[1,32] The prevalence of FP disease has reached epizootic proportions in several green turtle populations.[1,33] FP seems to be primarily a disease of juvenile green turtles following their migration to near-shore habitats.[34] Although FP was once identified as a major cause of green turtle strandings in Hawaii, more recent studies show that prevalence of the disease in Hawaiian green turtles is now in decline.[8,35] Two plausible explanations for this include the development of herd immunity and the removal of a tumor-promoting environmental insult in the near-shore turtle foraging habitats.[8,36,37] FP prevalence seems to be more stable in green turtles in the southeastern United States, with very high prevalence in some areas of Florida, where FP is considered a major cause of mortality.[11] In other locations, FP prevalence is increasing, and it has recently been reported from numerous new localities in the Atlantic and Pacific Oceans.[16,38–40] In many studies, ChHV5 DNA has been detected via molecular techniques in tissue samples collected from marine turtles with FP and from turtle populations in which FP prevalence is high.[29,41–43] ChHV5 prevalence independent of FP tumor prevalence has also been reported; for example, ChHV5 has been detected in normal skin biopsies from nontumored turtles in populations where FP is common.[43,44]

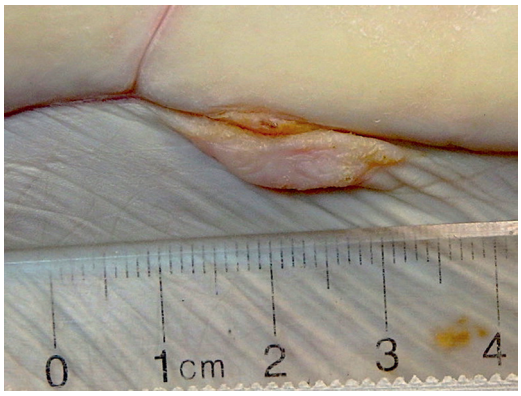
FP tumors are a proven source of infectious ChHV5 particles, and direct, horizontal transmission of ChHV5 was demonstrated in a series of infectivity trials.[20–22,28,36,45] The high prevalence of active ChHV5 infection in early tumors suggests that virus production and shedding predominantly



• **Figure 57.1** Severe multifocal fibropapillomatosis of a juvenile green turtle *(Chelonia mydas)* from the Florida Keys. Large verrucous tumors arise from and infiltrate the skin of the axial regions, ventral neck, front flippers, inguinal regions, cloaca, tail, and hind flippers as well as the posterior portion of the plastron. (Photo credit The Turtle Hospital.)

• **Figure 57.2** Fibropapilloma lesions on the left eye of a juvenile green turtle *(Chelonia mydas)* from the Florida Keys. Multilobulated verrucous tumors cover the epithelial surfaces of the conjunctiva, palpebrae, sclera, and cornea, partially obscuring the turtle's vision. Cutaneous tumors may also be seen on the left and right front flippers. (Photo credit The Turtle Hospital.)

occur early in the progression of FP disease.[26,28] It has also been postulated that ChHV5 transmission within a population may largely depend on a few highly infectious individuals with small tumors (<20 cm^2 surface area) permissive to viral production.[26] In areas where FP is endemic, viral shedding into the surrounding environment via sloughing of virally infected epidermal cells from FP tumors represents a key source of infection; in areas with high FP prevalence, epizootic transmission cycles are likely perpetuated in this way.[34] ChHV5 transmission may be magnified by mechanical vectors such as marine leeches *(Ozobranchus* spp.*)*.[30] Tissues other than cutaneous tumors may also be involved in ChHV5 replication and transmission cycles, as suggested



• **Figure 57.3** Flat, plaque-like cutaneous fibropapilloma lesion on the shell-skin interface of a juvenile green turtle *(Chelonia mydas)* from the Florida Keys. The tumor is smooth and sessile, with a broad base (2.0 cm × 0.6 cm). (Photo credit The Turtle Hospital.)

by a high prevalence of ChHV5 antibodies detected in areas of Florida with 0% FP prevalence.[46] In several studies, ChHV5 DNA has been identified via PCR in epidermal samples taken from nontumored turtles, suggesting that some turtles without gross evidence of FP disease are ChHV5-infected and may serve as a potential source of viral transmission.[43,44] To date, however, this assumption is unsupported by demonstration of infectious viral particles from apparently normal tissues. Because the full suite of potential FP transmission routes remains unknown, biosecurity is of utmost importance when handling marine turtles with FP. Thus measures of quarantine and prevention of transmission via fomites or personnel should be strictly implemented.[19,47]

Clinical Signs

FP tumors are typically proliferative masses that can occur anywhere on or within the turtle's body (see Fig. 57.1).[1,45,48] Morphologically, FP tumors may have a wide variety of gross appearances: flat plaques (Fig. 57.3), pedunculated, sessile, verrucous, smooth, or polypoid nodules, or a combination of multiple types. The number, color, and size of FP masses may vary widely, depending on tumor location and severity of disease. Secondary invaders such as bacteria and/or fungi readily infect ulcerated FP lesions.[27] The typical histologic description of cutaneous FP tumors includes papillary epidermal hyperplasia supported by broad fibrovascular stalks with a varying ratio of epidermal to dermal proliferation.[1,28] Lymphocytes and macrophages may be found at tumor margins and infiltrating tumors in moderate to marked numbers. Histologic evidence of clinical regression is observed in some tumors.[19] Visceral tumors are perceived as more chronic lesions that develop following cutaneous tumor proliferation.[1,13,28] Histologic descriptions of visceral tumors include fibromas, myxofibromas, and fibrosarcomas.[1,18,28,49]

Stranded and free-ranging sea turtles with FP are often debilitated and/or cachectic. Severe FP has been associated

with various abnormalities in clinical pathology data, including anemia, leukopenia, lymphopenia, eosinopenia, and heterophilia.[32,48,50] Hypoproteinemia, hypocalcemia, hypoalbuminemia, and hyperglobulinemia may also be observed in green turtles with FP.[32,48–53] Suggestive of anemia of chronic disease and antigenic stimulation, these changes are compatible with the clinical presentation of FP. Turtles afflicted with severe FP often present with a number of associated comorbidities, including bacterial, fungal, and/or parasitic coinfections, ileus, buoyancy issues, and boat-strike trauma.[19]

Diagnosis

Although cutaneous FP may easily be recognized on gross examination, definitive diagnosis requires histopathology findings compatible with FP. Follow-up diagnosis of ChHV5 DNA using molecular techniques is also recommended. If possible, all rehabilitating turtles with FP should be imaged to rule out visceral tumors. Widely available imaging techniques include radiography and ultrasonography; however, small soft tissue masses may evade diagnosis with these techniques. Observation of suspicious internal lesions on imaging may be followed by laparoscopy and biopsy.[19] Endoscopic examination also has limitations, however: dorsal lung and extraparenchymal lesions may be missed, and endoscopic procedures are not recommended in severely debilitated turtles.[13,19] If available, computed tomography (CT) or magnetic resonance imaging (MRI) may be preferred, as these techniques do not require anesthesia and tend to be more accurate in identifying small internal tumors.[54,55]

ChHV5 infection may be inferred via identification of viral DNA in biological swabs using molecular diagnostic techniques. For example, ChHV5 DNA has been demonstrated in cloacal, oral, and ocular swabs taken from turtles with cutaneous FP lesions, although these sample types are not as sensitive for ChHV5 DNA as tumor and/or skin biopsies.[25,56] Suspected cases of ChHV5 infection must be confirmed via direct visualization of areas of herpesvirus morphogenesis using histopathology and/or TEM in combination with molecular diagnostics such as PCR, in situ hybridization, or immunohistochemistry. There are several validated and published PCR assays targeting different ChHV5 genes, although assays offered by commercial diagnostic laboratories may be less specific than those used in research.[14,25,29,30,34,41,42,57] Confirmatory sequencing of PCR amplicons is required for proper diagnosis of ChHV5 infection. A serologic immunoassay based on recombinant antigen has been validated for detecting antibodies to ChHV5 glycoprotein H but is not currently commercially available.[46]

Treatment

Supportive care is essential for the successful treatment of FP, as the overall health of the turtle may strongly affect the course of FP disease and host immunosuppression, stress, and comorbid conditions can lead to viral reactivation.[58] Supportive care of turtles with FP should include water of suitable quality and temperature, adequate and species-appropriate nutrition, fluid therapy, pain management, and treatment of secondary infections.[59] Antiviral therapeutics (e.g., L-lysine, acyclovir) may be used to supplement supportive care; however, to date no controlled studies have been performed on the efficacy of these treatments against FP lesions.[19]

Although evidence suggests that some marine turtles with less severe FP will undergo spontaneous FP lesion regression, this should not be expected in most cases.[13,19,60,61] Surgical excision is currently the most effective way to treat cutaneous, oral, and ocular FP lesions. Local or general anesthesia can be used, depending on the size, number, and degree of invasiveness of the tumors. Multiple tumor excisions tend to require general anesthesia. The technique of choice is carbon dioxide (CO_2) laser–mediated tumor removal; other options include sharp excision, cryosurgery, radioscalpel, electrochemotherapy, and electrocautery.[13,19,62] The CO_2 laser helps minimize hemorrhage to the tumor removal site as it simultaneously cauterizes and seals the excision site or sites while it cuts tissue.[13] Laser power, pulse rate, and handpiece size may be varied according to surface area extent and depth of the tumor or tumors. Plaque-like or broad-based FP lesions may be ablated using a lower power, whereas pedunculated or narrow-based tumors may be excised using a higher-power technique. Extreme care should be exercised in removing ocular lesions, including low power and low pulse rate, avoiding corneal tissue by ablating ocular tumors at an angle. Sutures are usually not needed, and tumor excision sites may be left open to heal by secondary intention; however, sutures may be required in the case of removal of a very deep tumor. Perioperative analgesics and antibiotics should be administered. Careful postoperative monitoring should be implemented after tumor removal, including dry-docking patients for up to 24-hours postsurgery. Cutaneous lesions may heal completely in as little as 12 weeks. It is acceptable to perform multiple tumor removal surgeries, and this may be preferred in turtles with large tumor burdens.[13] The number of tumor removal surgeries was not significantly related to prognosis in one study.[19] A 4- to 6-week period of healing should be allowed between surgeries.[13] An important caveat of tumor removal surgery is the possibility of tumor regrowth: one study found that 38.5% of green turtles that underwent tumor removal surgery experienced FP regrowth within an average of 36 days postsurgery.[19] Although regrown tumors can be surgically removed, it is best to avoid repeated cycles of tumor removal and regrowth. To help prevent tumor regrowth, a wide margin of apparently normal tissue should be included in tumor excision whenever possible, as normal skin surrounding tumors may serve as a source of ChHV5-infected cells.[41] The likelihood of tumor regrowth may also be reduced by lowering tank water temperatures by 2°C–5°C after tumor removal surgery to help prevent viral reactivation.[19]

Prognostic Indicators and Release Considerations

Tumor number, anatomic distribution, morphologic appearance, and progression, as well as overall body condition and severity of comorbid conditions should dictate triage criteria for FP-afflicted marine turtles. If left untreated (and in some cases regardless of treatment), certain tumor locations and morphologies are associated with poor case outcomes; these include visceral or intraocular tumors, tumors that have eroded thorough bony structures (e.g., carapace, plastron), and aggressive recurrent tumors.[13,19] Turtles with these lesions may be considered outright euthanasia candidates. Other tumor types are more easily treated and should be considered on a case-by-case basis, taking into account comorbid conditions, available resources and treatment options, and quarantine capabilities. Although in one study, green turtles with ocular tumors were significantly less likely to survive rehabilitation than turtles with tumors but without ocular tumors, turtles with less severe ocular tumors may be candidates for treatment if materials and trained personnel are available. In the same study, turtles with only flat, plaque-like FP lesions had a significantly better prognosis, including spontaneous lesion regression in more than 50% of cases, as compared with turtles with more verrucous-types of FP lesions.[19] In general, FP is considered more of an incidental finding in loggerhead turtles *(Caretta caretta)*, and rehabilitation efforts focused on loggerheads with FP may have a more favorable outcome than those focused on green turtles with FP.[20] Based on clinical findings, medical opinion, and permitting conditions, the attending veterinarian should determine intake criteria, euthanasia candidacy, and release criteria for turtles with FP.

To be eligible for release, a turtle must be deemed capable of survival in its current condition. As long as the turtle is clinically stable and the overall assessment of survival following release is favorable, the presence of FP lesions should not prevent the release of rehabilitated turtles. It is recommended that green turtles be rehabilitated and released as quickly as possible, because the stress of captivity may contribute to FP tumor development de novo or exacerbate cycles of tumor removal and regrowth in ChHV5-infected turtles.[19] Thus reduction of tumor burden and rehabilitation to a clinically stable condition, including treatment of any comorbid conditions, are acceptable goals in preparing a turtle for release. The risk of introducing ChHV5 into naive wild turtle aggregations via clinical or subclinical carriers may be reduced by releasing rehabilitated turtles within the same geographic area from which they were recovered. Marine turtle rehabilitation programs should take into account FP prevalence within local wild marine turtle populations when evaluating resources and developing rehabilitation goals.

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