Nalla's No-Good Nodule

by

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Introduction

Squamous cell carcinoma (SCC) is the second most common equine skin tumor.[3,5,10,11,13] The tumor is commonly associated with squamous epithelium of non-pigmented mucocutaneous junctions.[3,5,8,10-13] Multiple predisposing factors have been associated with the pathogenesis of this carcinoma. These factors include unpigmented skin, chronic ultraviolet light exposure, equine papilloma virus-2, chronic skin irritation, and carcinogens such as smegma.[3,8,10,13] The tumor has a variety of behaviors, ranging from locally invasive and slow growing, to highly metastatic; however, metastasis is rare.[3,5,8,11,13] A definitive diagnosis is achieved using histopathology of a biopsy sample.[3,5,8,10,13] There are numerous treatment options available, including surgical excision, chemotherapy, photodynamic therapy, cryotherapy, and others; however, a combination of treatments is recommended.[3,8,10,13] The choice of therapy will vary depending on the location and severity of the tumor.

History and Presentation

Nalla is a 21-year-old Tennessee Walking Horse mare that presented on September 30, 2020 for suspected SCC on her vulva. Her owners first noticed multiple lumps on her vulva three months prior to presentation. The owners reported no change in her demeanor; however, they believed that one of the bumps has grown larger in the past few months. She was on a diet of half 12% protein grain and half alfalfa pellets. Her last Coggins test was performed in April 2020 and her vaccines were not up to date. No medication nor diagnostic tests were prescribed or performed by the referring veterinarian.

On initial presentation, Nalla was bright, alert, and responsive. She weighed 1045 pounds and had a body condition score of 6 out of 9. Her vital parameters were within normal limits,

revealing a heart rate of 48 beats per minute, respiratory rate of 16 breaths per minutes, and a rectal temperature of 100.3 degrees Fahrenheit. Her mucous membranes were pink and moist, with a capillary refill time of less than 2 seconds. Digital pulses were normal in all four feet. Her gastrointestinal motility was hypomotile in the left upper quadrant and within normal limits in the other three quadrants. On auscultation, normal bronchovesicular sounds were heard with no murmurs or arrhythmias. Her vulva had several tumors on her labia with the right side being the largest. On her left labia, there was a pink, firm superficial mass measuring 1.5 x 1 x 0.5 cm. On her right labia, there was a multilobulated mass measuring 4 x 3 x 2 cm. There were also four peri-vulvar small, raised, circular approximately 1 x 0.5 cm sized tumors. The remainder of her physical exam was within normal limits.

Diagnostic Approach

Although the clinical presentation of a lesion may be suggestive of SCC, histopathology is required for definitive diagnosis.[3,5,8,10,13] The sample should include both suspected neoplastic and normal appearing tissue.[10,13] The tissue should be stained using hematoxylin and eosin and observed using light microscopy.[10,13] If regional lymphadenopathy is suspected, a sample should be taken using fine needle aspirates, a biopsy instrument, or by removing the whole lymph node en bloc.[13] Normally, squamous epithelial tumor cells are well-differentiated and may progression through several layers of polyhedral basal cells.[11] These tumors are often diagnosed by histopathological findings of epithelial cell atypia and disorganization.[11]

Depending on the severity of the tumor, histologically, the changes of the cells can vary. Well-differentiated tumors, being confined to the epithelium, are considered in situ carcinomas/intraepidermal atypia.[11] These types of tumors transform from normal tissue to

epithelial hyperplasia.[11,13] From there, the tissue becomes neoplastic and the following histopathologic findings are commonly seen; parakeratotic hyperkeratosis, keratin pearls, and basal cell atypia/actinic keratosis.[10,11] As the pathology progresses, clumped chromatin, high nuclear to cytoplasmic ratio, prominent nucleoli, and atypical mitotic figures with variable mitotic rates can be seen.[11,13] SCC in horses is not ordinarily graded by pathologists, however multiple grading systems have been documented in the horse.[8,11,13] The grading system has three levels based on the depth of invasion and the degree of changed pathology of the cells.[11]

Nalla was sedated intravenously using butorphanol and detomidine prior to surgical excision. Three major sites were excised from her vulva and submitted for histopathology. The samples were stained with hematoxylin and eosin, and all exhibited the same characteristics under light microscopy. Sections of tissue have transitioned from normal appearance to hyperplastic, and then to neoplastic. In the areas with neoplastic changes, cells have divided deep into the dermis. Within these dividing cells, variably sized keratin pearls have formed. In the same slide, neoplastic cells have invaded the lumen of a vessel, indicating metastasis.

Pathophysiology

Any chronic irritation has the potential to promote neoplastic transformation of the epithelium into SCC.[4,5] Some of these irritants, or risk factors, include, ultraviolet light, chronic irritation or infection, equine papillomavirus-2, smegma, and other carcinogens that may cause genetic mutation.[4,5,8,10,11,13] Chronic ultraviolet/solar radiation is one of the most well-known factors, especially UV-B light.[4,11,13]

The specifics of the cell cycle, including replication, repair, restriction, and apoptosis, are controlled by the p53 tumor suppressor gene.[8,11] When the horse is chronically exposed to a

"carcinogen" or irritant, especially ultraviolet radiation, the p53 gene becomes susceptible to mutation, and consequently loses function.[4,8,11] This causes genetic instability and allows the epithelial cells to mutate.[8] Further mutation causes cells to shift from an in-situ carcinoma to a state where the tumor penetrates through the basement membrane, giving rise to potential metastasis.[8] This p53 mutation is mostly seen with ocular SCC.[4]

For genital SCC, a viral etiology has been proposed since there is an association between high-risk human papillomavirus causing genitalia cancers, and equine genital SCC.[12] Histologically, equine genital SCC precursor lesions look similar to high-risk human papillomavirus genital lesions.[12] The life cycle of papillomaviruses has been linked to the differentiation of cells within the epidermis.[12] These viruses are species-specific and carry a tropism for specific cells, such as cutaneous and mucosal keratinocytes.[12]

Equus caballus papillomavirus 2 (EcPV2) DNA has been detected in genital SCC lesions in multiple studies.[4,8,10,11,13] In multiple institutional and international screening studies, EcPV2 DNA and transcripts have been isolated from equine genital SCC and precursor lesions by PCR.[1,12] In most of the studies, an almost 100% correlation between genital SCC and EcPVR2 has been detected.[1,12] Each study also compared virus isolation from healthy individuals.[1,12] None of the studies were able to isolate the virus from ocular forms of SCC.[12] Overall, EcPV2 was detected in the majority of the carcinoma lesions and at most 10% from the control groups.[1,12]

Treatment and Management

There are a variety of treatment options available for SCC. The more common modalities include conventional surgical excision, cryotherapy, chemotherapy, hyperthermia, radiation, and

photodynamic therapy.[3,8,10,13] For Nalla's case, surgical excision with post-operative photodynamic therapy were used adjunctively. Surgical excision is most successful when performed early and should have margins of 0.5-1.0 cm but may be difficult based on tumor location and size.[13] Adjunctive treatments are commonly used following debulking.[5,8,10,13]

Photodynamic therapy combines a light source, oxygen, and a photoactive drug to create direct destruction of tumor cells, and indirect damage to the tumor vasculature.[2,6,7,9] This photoactive drug, or photosensitizer, can be administered in multiple ways: systemically (orally or intravenously) or locally (topically or via injection into the tumor bed).[2,6,9] Once the photoactive drug is accumulated within the tumor, it can only be activated via a specific wavelength of light.[2,7,9] The light source's wavelength is directly proportional to tissue penetration, with 630 nm being the least intense.[2,9] The most commonly used light sources are diode lasers.[2,7]

The exact mechanism of the drug's localization within neoplastic tissue is not well understood, however, it is hypothesized that tumor factors, such as leaky vasculature, high lipid content, etc., aid in the uptake and retention of the drug.[7,9] Once the light excites the photosensitizer, free radicals are produced, irreversible oxidation occurs, and a strong inflammatory response is induced.[7,9,13] Free radical production and oxidation are respectively referred to as "type-1 reaction" and "type-2 reaction".[7,9] Once the photoactive drug absorbs photons from the light source, it raises its energy state to an excited singlet or triplet state and can provoke one or both reactions.[7,9] Type 1 reactions occur from the photoactive drug interacting with molecules nearby the specific tissue via electron exchange, ultimately producing free radicals.[7,9] Type 2 reactions occur when the photoactive drug interacts with molecular oxygen, creating a reactive oxygen species.[7,9] Most studies done on photodynamic therapies have

been associated with small animals or humans, but the consensus is that side effects are rarely seen.[2,7] Small animals have experienced hyperemia, edema, pruritus and cyanosis at the targeted area.[2] At the doses used clinically, photodynamic therapy has not been documented to be mutagenic or carcinogenic.[7]

This combination of free radical production and oxidation with inflammatory mediators' affect different cellular components such as mitochondria, lysosomes, and plasma membranes, leading to apoptosis and necrosis of the tissue.[7] The tumor vasculature is also affected by the oxidative damage and assists in provoking tumor death.[7,9] Photodynamic therapy causes multiple pathways that lead to tumor death. The damaged lysosomes have acids and hydrolases that leak out and hasten the degradation of other cellular components.[7] The therapy also inhibits different mitochondrial proteins required to sustain the inner mitochondrial membrane.[7] The permeability transition pore, a protein complex on the membrane is targeted and opened, allowing small molecules to enter and exit the matrix.[7] These molecules generate swelling of the mitochondria and ultimately disrupt the membrane potential.[7] The treatment also plays a role in vascular destruction.[7] Microscopically, the vessels in the targeted area of the therapy have endothelial damage associated with platelet aggregation and are vasoconstricted.[7] Tumor destruction is further exacerbated by the body's natural inflammatory response, due to the release if metabolites that aid in accelerating phospholipid degradation.[7]

The day after Nalla's surgical debulking, the tumor beds were treated with indocyanine green, a photoactive drug, administered intradermally. Stay sutures were placed on the periphery of the surgical site to assist with tissue handling. Surgical excision is recommended prior to topical application due to the limited depth of the tissue that the photosensitizer can reach.[2] It is recommended to have a homogenous light distribution once the drug has penetrated the area.[2,6] A

diode laser is the most common, but some studies have reported using a light emitting diode, or LED.[2,7] An ARC FOX laser, operating at 810 nm, was used on Nalla's surgical site. Each of the three targeted areas was divided into four quadrants, with each quadrant receiving 90J at 500mW until we reached three rounds, each totaling 360J. The laser was then increased to 2.0W for the area on the left labia, and 2.5W for the area on the right labia due to their size and hemorrhage and was done until the area was smoking and looked charred. The area was left open to heal by second intention.

On October 1, 2021, Nalla was discharged from our equine hospital. The owners were instructed to give three days' worth of flunixin meglumine (Banamine). Either one 1000-pound dose by mouth once daily, or 10 milliliters of injectable form by mouth once daily. Her owners were instructed to reduce sun exposure by keeping her in a stall during peak sunlight hours, using UV protectant fly sheets, and applying SPF 40 sunblock or greater topically daily around the eyes and vulva. Since recurrence of SCC is common, we recommended Nalla's skin be monitored weekly for signs of developing tumors. A recheck was recommended in 4-6 weeks; however, Nalla did not return for a recheck appointment. In July 2021, Nalla returned to our facilities for assessment and treatment of new growths on her vulva. She had a 1 cm x 1 cm superficial growth on the left dorsal aspect of her vulva and an approximately 1 mm minimally raised area of white tissue on the mid-ventral aspect of her left vulva. Surgical excision of both areas and photodynamic therapy were performed. She was given flunixin meglumine (Banamine) with the same instructions as the first appointment, and 5-fluorouracil cream, a topical chemotherapy commonly used to treat small SCC lesions, to go home. The 5-fluorouracil was to be used after her incision completely healed, and on precancerous looking lesions every 1-2 weeks for 4 months.

Conclusion

Squamous cell carcinoma is a locally invasive skin tumor with a rare likelihood of metastasis. With chronic solar radiation being the most common etiology, preventative measures should be taken to reduce sunlight exposure, especially in horses with unpigmented skin. These measures can include applying sunblock topically, using UV protectant sheets, and supplying shaded areas. There are a variety of treatment modalities that have been proven successful for equine SCC. Due to its rare side effects and general safety of the treatment, photodynamic therapy provides many benefits to the patient, the owner, and veterinarian. The limiting factors of the therapy would be the size and location of the tumor, the cost of the equipment, and having a proper facility to house the equipment if needed. One of the restrictions of the treatment would be if the patient's disease had already metastasized. With photodynamic therapy's precise application, it has not been proven to be effective in the treatment of invading tumors. Nalla's histopathology showed prominent vascular invasion and tumor recurrence occurred 10 months later. With SCC's low chance of metastasis, and adequate monitoring of a patient's skin, photodynamic therapy plays a valuable role in the treatment of SCC.

References

1. Bogaert, L., Willemsen, A., Vanderstraeten, E., Bracho, M. A., Baere, C. D., Bravo, I. G., & Martens, A. (2012, February 11). *EcPV2 DNA in equine genital squamous cell carcinomas and normal genital mucosa*. Veterinary Microbiology. Retrieved January 20, 2022, from

https://www.sciencedirect.com/science/article/pii/S0378113512001150?casa_token=Onyfa-mAuWIAAAAA%3AiGxSRRK2_LwsmEAiJet-dSskVBaEv1RayJkO3VTOXVgkcwMZG0mASd1gw2rOoHTU_NkTqgKZmA

2. Buchholz, J., & Walt, H. (2013, July 1). *Veterinary photodynamic therapy: A Review*. Photodiagnosis and Photodynamic Therapy. Retrieved January 20, 2022, from https://www.sciencedirect.com/science/article/pii/S157210001300077X
3. Eddy, A. (2019). *Equine Cutaneous Neoplasia and Cutaneous Habranemiasis*. *Equine Medicine and Surgery 1*.
4. Elce, Y. A. (2009). The aetiopathogenesis of squamous cell carcinomas in horses. Where are we? *Equine Veterinary Education: Clinical Commentary*, 17–18. https://doi.org/10.1111/eve.2014.26.issue-5
5. Giuliano, E. A. (2011, March 3). *Equine periocular neoplasia: Current concepts in AETIOPATHOGENESIS and emerging treatment modalities*. British Equine Veterinary Association. Retrieved January 20, 2022, from https://beva.onlinelibrary.wiley.com/doi/10.1111/j.2042-3306.2010.tb05629.x
6. Giuliano, E. A., MacDonald, I., McCaw, D. L., Dougherty, T. J., Klauss, G., Ota, J., Pearce, J. W., & Johnson, P. J. (2008). *Photodynamic therapy for the treatment of periocular squamous cell carcinoma in horses: A pilot study*. Veterinary ophthalmology. Retrieved January 20, 2022, from https://pubmed.ncbi.nlm.nih.gov/19046267/
7. Giuliano, E. A., Ota, J., & Tucker, S. A. (2007). *Photodynamic therapy: Basic principles and potential uses for the veterinary ophthalmologist*. Veterinary ophthalmology. Retrieved January 20, 2022, from https://pubmed.ncbi.nlm.nih.gov/17970993/

8. Knottenbelt, D. C., & Croft, J. S. (2018, April 25). *Cutaneous squamous cell carcinoma (SCC): "what's the problem?"*. British Equine Veterinary Association. Retrieved January 19, 2022, from https://beva.onlinelibrary.wiley.com/doi/10.1111/eve.12926
9. Lucroy, M. D. (2005, March 1). *Photodynamic therapy for companion animals with cancer*. Veterinary Clinics of North America: Small Animal Practice. Retrieved January 20, 2022, from https://www.sciencedirect.com/science/article/pii/S0195561602000116?via%3Dihub
10. Poore, L., Smit, Y., Duncan, N., & Williams, J. (2018, March 9). *Surgical resection of a squamous cell carcinoma in the perianal region of a 25-year-old crossbred American paint gelding using Sharp Surgical Excision, laser excision and chemotherapy*. British Equine Veterinary Association. Retrieved January 19, 2022, from https://beva.onlinelibrary.wiley.com/doi/10.1111/eve.12906
11. Snalune, K. L., Knottenbelt, D. C., & Patterson-Kane, J. C. (2015). Squamous cell carcinoma. In *Clinical equine oncology* (pp. 220–229). essay, Elsevier.
12. Sykora, S., & Brandt, S. (2017, May 31). *Papillomavirus infection and squamous cell carcinoma in horses*. The Veterinary Journal. Retrieved January 20, 2022, from https://www.sciencedirect.com/science/article/pii/S1090023317301041?casa_token=G-jpyvbmcOQAAAAA%3AwBCSfnInakp7djZmFdlbkdYfwvEAPP9U9y_uTuXPAjXBQl0PME3UHapBVMZ6cpH_Z8vhm1THtw
13. Taylor, S., & Haldorson, G. (2012, September 26). *A review of equine mucocutaneous squamous cell carcinoma*. British Equine Veterinary Association. Retrieved January 19, 2022, from https://beva.onlinelibrary.wiley.com/doi/10.1111/j.2042-3292.2012.00457.x