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Review Article

A Critical Review of the risk factors associated with Canine Squamous Cell Carcinoma development

Derick Santana Alves[1], Alexandre Matheus Baesso Cavalca[2], Carlos Eduardo Fonseca Alves[1,2]*.

[1] Institute of Health Science, Paulista University – UNIP, Bauru, SP, Brazil
[2]Department of Veterinary Surgery and Animal Reproduction, São Paulo State University – UNESP, Botucatu, São Paulo, Brazil

***Corresponding author:** carlos.e.alves@unesp.br

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Abstract

Squamous cell carcinoma (SCC) is a common neoplastic skin disease that is highly prevalent in tropical countries. As the skin has a variety of cells, overexposure to environmental factors, such as ultraviolet light, can affect this organ, resulting in malignancies, such as cutaneous SCC, hemangioma, and hemangiosarcoma. SCC arises from keratinocytes in the skin and is locally invasive with low metastatic rates, commonly affecting unpigmented skin in sites with high exposure to sunlight, such as ventral regions. SCC has a variable etiology that is not well understood. Therefore, literature review aimed to critically evaluate the risk factors involved in the SCC development.

Key words: Canine, Actin keratosis, Papilloma virus, Oncoproteins, Skin, Ultraviolet radiation.

Introduction

The skin is the largest organ in the body and is the most common neoplastic site in different species. The skin can be affected by several tumors as the skin is composed of a variety of different cell types that can undergo malignant transformation and is exposed to environmental factors, inducing a high rate of mitotic division and cellular regeneration (6). Squamous cell carcinoma (SCC), mast cell tumor (MCT), and hemangiosarcoma are the most common skin tumors. Interestingly, the number of dogs affected by tumors has been growing in recent years due to increased life expectancy, advances in animal nutrition, vaccination, preventive therapies, and better animal healthcare (44).

SCC is a malignant neoplasm with variable clinical appearances. It also represents one of the most common skin neoplasms in dogs in tropical countries. SCC arises from keratinocytes in the skin epithelium, with high local invasiveness and low metastatic rates. They are formed in glabrous regions, unpigmented, or lightly pigmented skin (15, 44). SCC has a multifactorial etiology, including genetic predisposition, exposure to physical and chemical factors, exposure to ultraviolet (UV) light, and infection by papilloma virus (PV), as suggested by the genomic differences and mutations in the E5, E6, and E7 genes. Proteins have also been implicated in the etiology of SCC (9, 15, 44). Clinically, it can present with single or multiple lesions of variable size, such as papillae, scales, or fungal masses, alopecia, ulceration, crusting, erythema or hemorrhage (15, 44).

To diagnose SCC, a cytopathology exam can be requested to enable the retrieval of quick results. This exam is also associated with an easy execution, low price, and noninvasive techniques; a needle, microscopy slide, and syringe are the materials required for this exam. However, histopathological examination can provide a definitive diagnosis (47).

The main treatment for SCC is surgical removal, with or without chemotherapy, as suggested for metastatic cases. Radiotherapy and electrochemotherapy are the most frequently cited treatment methods in the literature. An alternative to these methods is cryosurgery for small tumors and photodynamic therapy, plesiotherapy and diathermia ere also previously described (44). The prognosis of SCC depends on the location and clinical stage at diagnosis (favorable when diagnosed early and with complete surgical exeresis) (15, 44). Due to the importance of SCC in dogs, this review aimed to critically discuss the etiology and the carcinogenic and molecular factors associated with the development of SCC.

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Etiology and Epidemiology

Skin neoplasms are common in tropical countries due to chronic exposure to sunlight (15, 47). A previous study conducted in Brazil (6) revealed that the estimated incidence of skin neoplasm in dogs is 728 of 100.000 cases each year, with SCC as the second most commonly diagnosed skin neoplasm, representing 15.3% of the cases. The breeds most affected by SCC include Pit-bull, Dalmatian, Terriers, Beagles, Schnauzer, Basset Hound, and Collie (15, 44). In another study, mixed-breed dogs were found to be most affected by SCC (36%), followed by American Pit-bull (7). However, as sunlight exposure is one of the most important risk factors, these breeds are more likely to have unpigmented skin (12).

As sunlight exposure is one of the most important factors, the affected breeds are mainly those with lightly pigmented or non-pigmented skin. One fact that supports this hypothesis is the lesion location, with the ventral abdominal, ears, and eyelids, which are regions with light skin and fur, as the most affected areas (15, 44). The skin has a high rate of cell renewal which makes it very susceptible to mutations, due to direct exposure to oncogenic factors, such as the sunlight. SCC more often occurs when the skin is damaged by sunlight, which is preceded by actinic keratosis (AK) (4). The electromagnetic spectrum is composed of different types of waves, and the longer the length, the greater the penetration power. UV radiation is divided into UVA, UVB, and UVC; UVB is known to cause erythema, pigmentation, and changes that predispose the skin to cancer development. The intensity of the radiation as well as the length depend on the altitude, latitude, season, atmospheric condition, and time of day (51).

The most accepted cause of SCC is exposure to UV light. UV radiation acts as a carcinogen, causing photochemical reactions that activate inflammatory pathways that change the immune system and directly attack DNA. This event results in the inappropriate repair of photoproducts from DNA, mutations in regulatory genes, and expansion by cloning. Excessive exposure to sunlight causes AK, a preneoplastic condition (15, 44). Costa et al. (12) highlighted that AK and SCC are similar conditions and are considered to be the same disease at different stages of differentiation (Fig. 1). UV rays cause mutations in p53 and p16, which are tumor suppressor genes. p16 was identified in 100% of AK cases. AK precedes SCC induced by UV radiation. Beyond its influence on DNA, AK can cause a transient immunosuppressive effect in the skin, which affects the normal function of Langerhans cells (44).

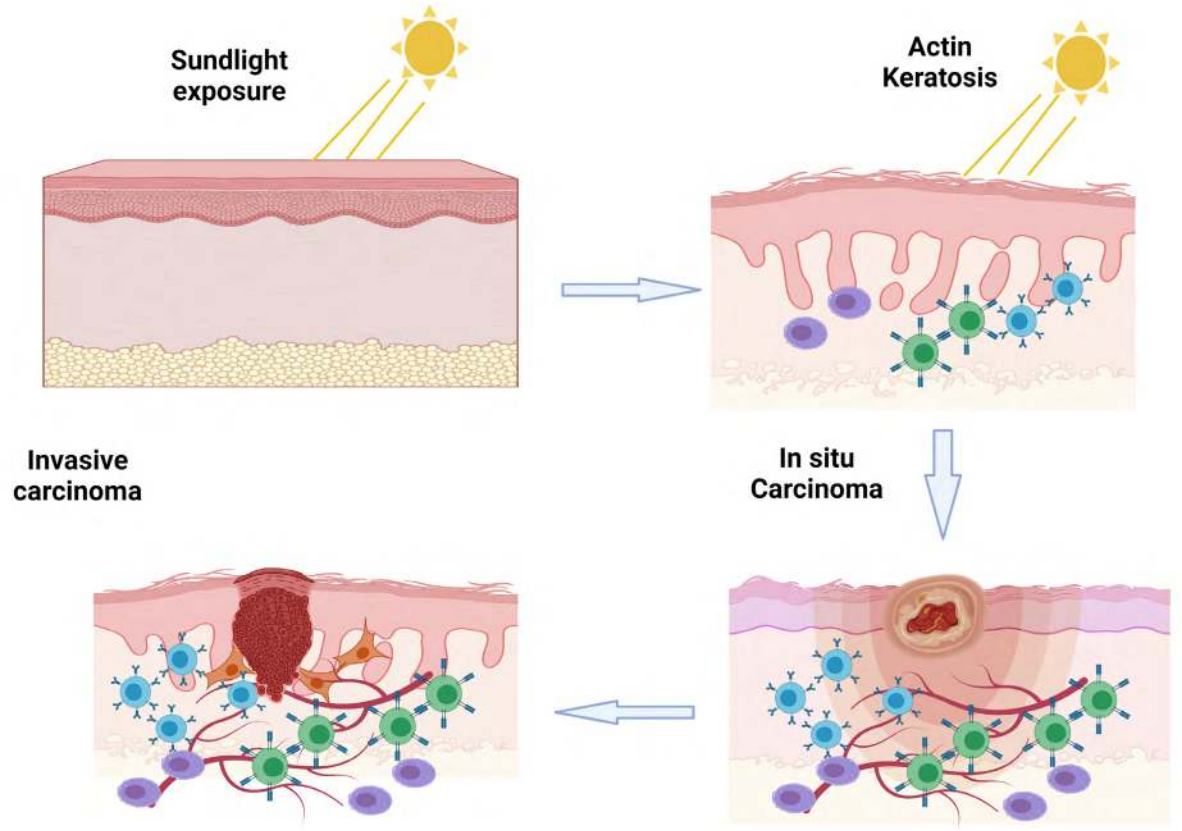

Figure 1. Development of actinic keratosis due to chronic sunlight exposure. Chronic sunlight exposure induces cell damage, inflammatory cell infiltration, morphological and biochemical modifications, causing the development of actin keratosis. With the continuous sunlight exposure, a chronic inflammatory process induces angiogenesis and progression of in situ carcinoma. Therefore, with continuous UV exposure and chronic cellular damage, an invasive carcinoma develops.

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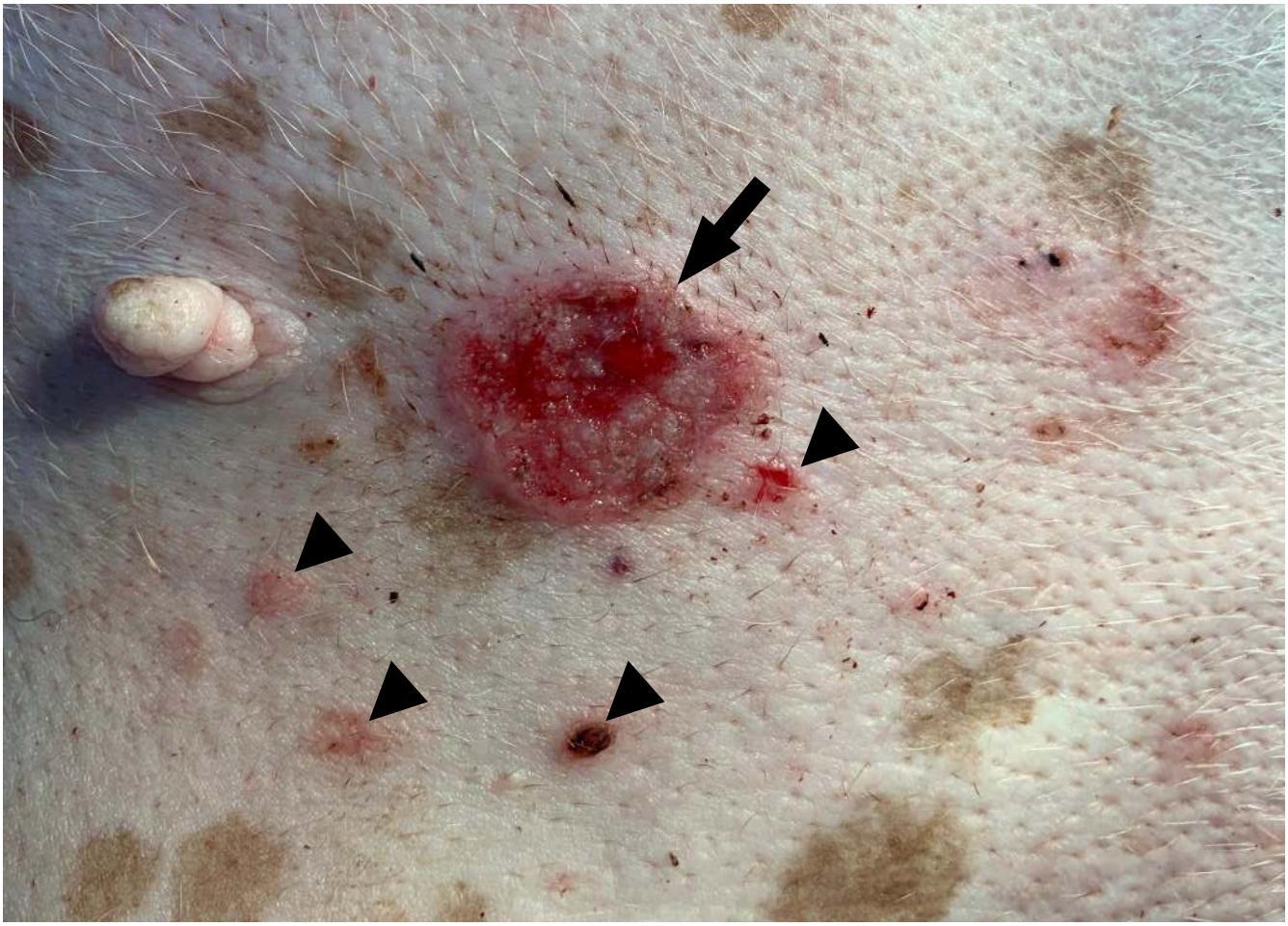

Figure 2. Cutaneous squamous cell carcinoma (SCC) (arrow) developed in a dog with a history of chronic sunlight exposure. Note the erythematous small areas of actinic keratosis surrounding the SCC (arrowhead). Usually, lesions occur in abdominal region.

Actinic keratosis

Actinic dermatitis or AK is an environmental disease that associated with chronic sunlight exposure and cellular damage, which involves the epidermis, superficial blood vessels, and deep vascular plexus (5). Chronic actinic dermatitis is one of the hypotheses for the development of skin neoplasms, such as SCC, that will be discussed (Fig. 2).

Some pathologists suggest that there is no clinical difference between AK and SCC; this is because the SCC progress from AK and the normal skin of dogs with SCC or AK can display alterations induced by exposure to UV rays (12, 33). Histopathological examination is necessary to confirm disease diagnosis and differentiate between lesions. In clinical evaluation, the skin can present focal erythema, scabs, flaking, erosion, or ulcerations (33).

The lesions appear in areas chronically affected by sunlight in non-pigmented regions with glabrous areas or a small amount of hair, such as the ventral and lateral abdomen and flank (34). The same condition for the appearance of SCC has been discussed previously. UV exposure causes morphological changes in Langerhans cells and influences cytokine release. UVA rays cause photooxidative stress, which indirectly causes mutations in DNA. UVB acts directly on thymine dimer formation and transitions in DNA or RNA. The absence of a regulatory mechanism as a repair mechanism can initiate the mutation in keratinocytes that progress to AK (12, 34).

The literature suggests that UV light can cause mutations in the p53 gene. As the epidermal cells are exposed to UV light in regions where the precursor cells are located, the DNA can absorb UV light, causing mutations in some genes.. In human patients with AK, the incidence of mutations in p53 was found to be approximately 75%–80%. Other studies on p53 revealed mutations in this gene in keratinocytes with AK or SCC. Inactivation of the p53 gene causes cell proliferation 12.

Costa (12) revealed that the main difference between AK and SCC is the invasion of the basal membrane by neoplastic cells. Therefore, atypical mitotic figures combined with histopathological, clinical, and immunohistochemical changes clarify the similarities between AK and SCC, suggesting that both are the same disease in different stages.

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Table 1. Details of previous studies that investigated the presence of Papillomavirus in canines with actin keratosis or squamous cell carcinoma.

Author	Objective	Methods	Results
Zaugg et al (14).	Detect a broad spectrum of DNAs of PV in samples from three forms of SCC and sequence the amplified DNA.	PCR using formalin-fixed paraffin-embedded (FFPE) samples.	Discovered PV DNA in 9 of the 42 samples of canine SCC with broad-range PCR and 1 of the 42 with narrow-range PCR.
Waropastrakul et al. (15)	PVs were hypothesized to act as a cofactor in canine UV induced SCCs.	DNA was extracted from FFPE tissues. PCR was used to amplify and discover the PV DNA.	PCR amplified the PV DNA from both viral plaques. PV DNA was only amplified in one of the 20 cutaneous SCC samples.
Munday et al. (16)	Compare the presence of PV DNA in subungual SCC to that in non-SCC digit lesions.	Detection of PV DNA was confirmed by amplifying glyceraldehyde-3-phosphate dehydrogenase gene. Two PCR primer sets were used to amplify DNA from samples.	The primer set only amplified PV DNA from positive control samples. None of the samples contained DNA that could be amplified by consensus primers
Munday et al. (17)	Describe a case of a dog with invasive SCC and multiple situ carcinomas of the oral cavity.	PV DNA was amplified by PCR using two consensus primers. Immunohistochemical staining was used to detect the PV antigen	Immunohistochemical staining confirmed the presence of the PV antigen. PCR amplified the DNA using both consensus primers.
Luff et al. (18)	Describe a case of progression of pigmented plaques to invasive and metastatic SCC associated with 2 canine PV.	The sample was fixed in formalin 10%, for immunohistochemistry. The PV antigen was detected in fixed paraffin-embedded tissue samples. For detection of canine papillomavirus (CPV) DNA, PCR was used.	Immunohistochemistry revealed keratinocytes from pigmented plaques with strong nuclear immunoreactivity consistent with viral infection. PCR revealed PV DNA sequences from 2 different CPVs.
Sabattinie et al. (19)	Investigate the relationship between p16 expression and PV DNA presence in a series of canine SCC retrieved from different anatomical locations.	PCR was performed using FFPE samples of SCC.	PV DNA was detected in 3 of the 52 samples.
Ibarra et al.(13)	Discuss a potential cause of chronic infection and other factors that contributed to the development of oral SCC in a 3-year-old dog.	An FFPE sample of SCC was tested. PCR was performed in 2 laboratories with different primer sets to detect CPV-1 and CPV17.	A CPV-1 sequence DNA was detected in the samples by the 2 different laboratories.
Reis et al.(20)	Evaluate the variability of the PV L1 capsid gene lesions in naturally infected dogs.	Samples were divided: one half was FFPE for histopathological examination, and the other half was frozen for DNA extraction. The CPV L1 gene was also assessed using PCR.	Five different mutations were found in the samples. One CPV1 sequence, from an oral SCC sample, had a highly destabilizing substitution in the L1 protein.
Alves et al. (21)	A case report of a dog with pigmented plaques induced by CPV16 that progressed to in situ SCC.	Samples of tissue lesions were divided: half was fixed in formalin for histopathology exam and in situ hybridization. The other half was stored frozen and subjected to PCR and HTS.	CPV was detected by PCR using primer pairs.
Orbell et al. (22).	A case report of a dog that developed viral plaques, basal cell carcinomas, SCC, and trichoblastoma.	Detection of PV was achieved by PCR, with multiple consensus primers. The DNA was extracted from cores of 70% Ethanol-Fixed Paraffin-embedded tissue sample.	CPV-3 was detected in multiple lesions.
Chan et al. (5)	Identify the viral DNA of PV in FFPE tissues diagnosed as SCC or Papilloma using general primers.	DNA was extracted from FFPE tissues and identified using PCR targeting the E1 and L1 gene. The positive samples were submitted for immunohistochemistry analyses.	PV DNA was detected in 11 FFPE samples and four oral tissues. CPV-1, 2, and 6 were detected in benign lesions using PCR and confirmed with IHC. CPV-9 and 15 were first detected in the SCC of dogs; CPV 16 was often detected in SCC samples.

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Canine Papillomavirus

Papillomaviruses are non-enveloped, icosahedral viruses that infect the stratified squamous epithelium of many mammals. Propagated by direct or indirect contact, the PV can proliferate upon contact with the mucocutaneous epithelium. The presence of microabrasions allows infection of basal cells, producing episomes (small number of circular PV DNA) within the cell, ultimately maintaining the basal cells as they replicate. The viral life cycle is complete only when the infected cell undergoes terminal differentiation. A total of 95% of lesions spontaneously regress within 2 to 4 weeks after becoming clinically evident (19, 26). PV infection has been suggested as a possible etiology of SCC in the literature. Studies on the DNA of PV in SCC have some similarities and contradictions in their reported results, as shown in Table 1.

Chang et al. (9) discussed the detection of PV in their work. Due to the abundance of genotypes of PV and high heterogeneity among the different types, detection has been a challenge. Further, no primer set can amplify all types of PV. In this research, the researchers used commonly used primer sets (CP4/5) to detect PV and to search for new types. CPVs 2, 3, 7, 16, and 17 were detected in SCC. In a normal life cycle, PV produces proteins that influence cell growth and differentiation (20, 38). CPVs 1 and 13 are commonly observed as non-neoplastic papilloma in the oral cavity (9). Some studies have reported an association between oral SCC and CPV 1 (19), and oral SCC and CPVs 2, 3, 7, 16, and 17'(21, 25). The genes of CPV 7, 12, and 16 were detected in the cutaneous SCC of dogs (9).

Munday et al. (25) assessed a Labrador male dog with an exophytic mass and 7 smaller raised, pale, roughened plaques within the right buccal gingiva. Histological examination of the plaque revealed neoplastic epithelial cell proliferation with PV cytopathic changes. DNA was extracted from the frozen half of gingival SCC and PCR was carried out for DNA amplification. Phylogenetic analyses revealed a 70% similarity between CPV-2 and CPV-7, with the 17th PV identified in domestic dogs. Therefore, CPV-17 infection could influence the development of neoplasms, but is rarely found in neoplasms in dogs.

Orbell et al. (32) reported that a 12 year-old dog with a history of multifocal PVPs developed multiple additional plaques with underlying nodes. These researchers also discussed the association between PV etiology and total DNA extracted from cores of 70% ethanol-fixed, paraffin-embedded tissue samples. These samples were collected from deep areas of the nodules to avoid asymptomatic PVs that may be present on the skin surface. Multiple consensus PCR primers were used to amplify PV DNA.

Ibarra et al. (19) described a case of a 3 year old neutered male Labrador retriever with nonregressive refractory oral CPV-1 infection that developed oral SCC after 18 months. The researchers discussed the PV life cycle (summarized in Table 2 below), based on the schedule proposed previously, and revealed that some stages develop in multiple layers of the epithelium, inducing macroscopic and microscopic changes. A finding in this case was the absence of cellular infiltrates in the histopathology reports, suggesting a deficiency in cellular immunity in the patient, in contrast to humoral immunity, which has no role in the resolution of infections. A second histopathology confirmed a well-differentiated but invasive SCC, with two PCR tests confirming the presence of CPV-1 DNA within the mass. It is also revealed the etiology of SCC. According to some researchers, the DNA of PV in the SCC is a result of incidental colonization of the virus or contamination of specimens during PCR testing (50).

Table 2. Canine papillomavirus life cycle summarized in relation to the epithelium layer with the life cycle phase and the interaction of the virus with the host cell.

Layer	Phase of the Life Cycle	Interaction
Squamous granular and suprabasal	Acquisition	Microlesions affect the oral mucosa, enabling PV to infect the keratocytes
Basal	Infection	The DNA of PV is mixed with the DNA of the host cell in the keratinocytes
Suprabasal	Multiplication	Mitosis of the infected cells occurs. PV spreads to adjacent cells
Granular layer	Viral expression	PV viral genes are activated, causing DNA replication
Squamous layer	Viral assembly	The DNA of PV becomes clustered, creating capsid proteins in differentiated keratinocytes.
Keratin Squames	Release	Viral particles embedded in keratin strands in cell dissolution are released, infecting another epithelium

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The oncogenic role of PV in SCC is hypothesized to be related to the proteins, E6 and E7, which induce unregulated cell replication and inhibit the host immune response to neoplasm. Another hypothesis is the inactivation of p53, which contributes to the progression of PV lesions into SCC (19).

According to the study by Luff et al. (21) with two Basenji dogs, the carcinogen induced by the PV is rare, and in most cases of SCC, does not arise from PV-induced precursor lesions. However, dogs with mutations in the common γ-chain can develop a papilloma that progresses to metastatic SCC. In the report, malignant transformation and progression of pigmented plaques were described, with an association of two different types of canine oral PV. The samples were assessed using PCR to differentiate the PV species. Immunosuppression and genetically based diseases are considered as predisposing factors for PV infections. Further, viral factors that can be associated with the risk of malignant transformation may exist. Nevertheless, this report was not conclusive due to the low number of dogs. More evidence regarding the transforming abilities of the viral oncogenes is thus required.

Zaugg et al. (52) revealed a fraction of canine SCC infected with PV, which is a genetic variety of canine PV. In their study, the PV DNA could be identified in 10 of the 42 canine SCC samples. However, the results obtained are not proof of the carcinogenic potential of the PV. The detection of other families of PV cells is important for further research. In canine, oral PV DNA is rarely present in the SCC samples as PCR cannot amplify the DNA of all PVs. Further, the use of formalin-fixed and paraffin-embedded (FFPE) tissue decreases the amplification rate of viral DNA.

As discussed in this review, it was difficult to amplify the DNA of PV cells on SCC by PCR (28). The FFPE process can damage DNA and prevent amplification (46, 49, 52). In Steinau's study (Steinau et al. 2011) only 62.7% to 73.3% of HPV-positive cases detected by qPCR remained positive after the FFPE procedure. Because of the large types of PV that can infect dogs and the novel PVs being described, primers that detect a wide range with more sensitivity could be used in the experiments. However, there is no primer set that can amplify all types of PV (16). The failure to amplify PV DNA could be related to a possible transient infection of the skin. This theory suggests that the viral antigen can cause mutations without PV DNA remaining detectible (46, 49).

Immunohistochemistry has also been used to detect intralesional viral antigens (9). However, this technique has limitations: an active replication stage by the PV antigen is needed (32, 46, 49) and the failure to detect PV using this method does not exclude the PV etiology (32). It is important to highlight that each technique has different limitations, and each limitation is considered in the study design. Performing only PCR is insufficient to associate a direct relationship between the presence of PV and tumor development. Other studies have reported that dogs are not a suitable animal model for High-Risk HPV-Induced Oral Cancer (35). Thus, the localization of PV is important for this association.

In human medicine, several studies have used PCR and in situ hybridization to reveal the location of the PV in neoplastic cell nuclei (17, 18, 36). Currently, our research group is carrying out studies on the PV in cancers of domestic animals. PCR-detecting virus sequences and positive immunohistochemistry (Fig. 3) or in situ hybridization in the nucleus of neoplastic cells can be considered the gold standard technique que associated papilloma virus infection and tumor development.

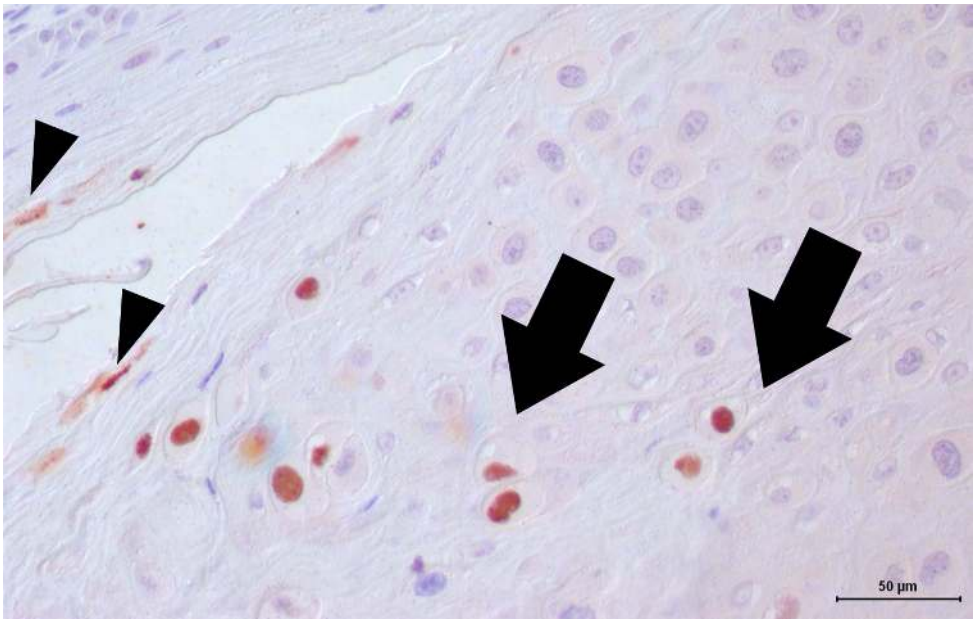

Figure 3. Immunohistochemistry of the papilloma virus proteins in canine squamous cell carcinoma from our research group. Positive nuclear expression was exhibited by neoplastic cells (arrows) and stromal adjacent cells (arrowhead). Thus, in a tissue sample, expression in both neoplastic or stromal cells could be expected; however, only expression in neoplastic cells could be considered to be associated with tumor development.

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Oncoproteins

The p53 tumor suppressor protein controls neoplasm growth, ensuring the stability and integrity of the cell and cell genome. It is a multifunctional protein that regulates the normal cell life cycle and mediates the response to genotoxic stress, senescence, differentiation, and cell death, where the loss of action results in cell proliferation and resistance to mechanisms of cell death and metastasis (10). The mutation or loss of the ***TP53*** gene in neoplastic cells maintains the viability of the cell by reducing the action of p53. In lesions developed because of physical, chemical, or biological agents, p53 acts to stop the mitotic process, enabling the cell can correct the mutation or induce apoptosis (3, 8). UV radiation can initiate carcinogenic development, inducing a mutation in TP53 and neoplasm development (23). Studies on the p53 protein can assist in the formulation of therapeutic agents for target proteins(10).

Cossi et al. (11) evaluated the expression of the p53 protein in corneal SCC and investigated its function in SCC development. Of the six samples, five were SCC and one was AK. However, p53 was found to be expressed in 100% of the samples. These researchers concluded that the expression of these proteins indicates their importance in the development of this neoplasm.

The $p16^{CDKN2A}$ tumor suppressor (p16) is a protein that inhibits the cell cycle and is frequently hypermethylated in precancerous oral lesions. Lesions with a hypermethylated p16 promoter tend to transform into oral cancers (1). In human oral cancer, the loss of p16 expression has been observed in oral lesions and primary tumors. The gene mutation, deletion of the homozygous gene, and hypermethylation of upstream CpG island regions are the mechanisms of inactivation (37).

In human cases of oncogenic PV and feline cases of cutaneous SCC, an association was found between p16 tumor suppressor protein (p16) immunoreactivity and the presence of PV DNA. Some studies observed different survival times in cats with p16 positive and negative nasal planum SCC, which suggests that p16 could be used as a prognostic indicator. Although p16 positive tumors were found in canine oral SCC studies, no PV DNA was detected, which suggests that increased p16 may not have been due to PV infection (24, 37). Sabattini et al. (46) detected PV DNA in only three of the 52 samples tested. In contrast to humans and felines, PV infection does not show a relationship with p16 immunostaining, which was observed in 20% of cases.

Ressel et al. (42) studied the expression of p63, a transcription factor responsible for the maintenance of the proliferative potential of epidermal stem cells during epidermal stratification and that also regulate the differentiation of epidermal keratinocytes by the cytoplasmic expression of intermediate filament CK5 in CPV and SCC. According to these researchers, the co-expression of p63 and CK5 immunostained epidermal keratinocytes represents a high proliferative capacity, similar to stem cells. Such finding suggests that transit-amplifying cells and keratinocytes may contribute to epidermal neoplasms in dogs (23).

Ressel et al. (40) also studied the expression of phosphorylated epidermal growth factor receptor (pEGFR) and phosphatase and tensin homologue (PTEN) in canine cutaneous papillomas and squamous cell carcinomas. The expression of pEGFR and PTEN was dysregulated in most samples, with overexpression of pEGFR and decreased expression of PTEN. This reports may facilitate the progression of cutaneous papillomas and squamous cell carcinomas involving PI3K/Akt/mTOR signalling pathway, a key regulator of cellular functions (39).

In cells with genetic lesions, the Bcl-2 protein contributes to the clonal expansion of the cell, interrupting apoptosis, which leads to immortalization (13). According to Dornelas et al. (13), in human SCC cases, the expression of Ki-67 and the lack of expression of Bcl-2 indicate an intense proliferative activity, where the expression of p53 and Bcl-2 in AK suggests apoptotic alterations resulting in cell immortalization. The expression of the Ki-67 antigen occurs in all phases of the cell life cycle, except in G0 or mitosis, which is a marker of cell proliferation. This antigen is also found in cells in the G1 phase to the M phase but is not found in differentiated cells.

Studies in humans have concluded that inflammation is associated with the progression of AK to SCC. Cyclooxygenase 2 (COX-2) is an induced form involved in the synthesis of inflammatory mediators. COX-2 is usually undetectable in tissues, thereby inducing tumor promoters, carcinogens, cytokines, and growth factors. Increased expression of COX-2 is observed in neoplasms and is involved in carcinogenesis. COX-2 induced by UV light exposure acts as a carcinogen in angiogenesis, apoptosis inhibition, immunomodulation, cancer cell metabolism, cell mobility, proliferation and invasion, conversion of pro-carcinogens to carcinogens, and metastasis capacity, ultimately inducing the increased expression of metalloproteinases (MMPs), which assist in the disruption of tissue barriers (12, 30, 43). The photocarcinogen is related to the production of reactive oxygen species and inflammatory prostaglandins by COX-2, which in association with those produced by the inflammatory cells induced by UV light, causes oxidative lesions in DNA (45).

Poggiani et al. (34) discussed the relation of COX-2 between samples of AK and SCC; however, immunohistochemical staining for COX-2 did not reveal any variation in cells of the SCC samples and AK samples. The results of this research suggest the involvement of COX-2 in the development of skin neoplasms affected by sunlight overexposure. Since COX-2 expression have been associated with development and progression in AK and SCC, COX-2 inhibitors could be used in clinical practice the treat both lesions.

Nagamine et al. (29) reported the role of evaluation immunohistochemical expression of E-cadherin, β-catenin, desmoglein, vimentin, and N-cadherin in histological invasive front grading and epithelial-mesenchymal transition in canine oral and cutaneous squamous cell carcinomas. In neoplastic cells, changes in adhesion molecule expression and transition from epithelial to mesenchymal phenotype are thought to be important in development of invasive behavior and imminent tumor. Therefore, the study suggested that E-cadherin and β-catenin and desmoglein as markers for predicting biological behavior of canine canine squamous cell carcinoma.

The reduced expression of E-cadherin affects cell adhesion, increases the risk of neoplastic progression, facilitates the differentiation of neoplastic cells, and increases the invasion and development of metastasis. The expression of E-cadherin was found to be low in the neoplastic epithelium and non-neoplastic epithelium. Further, Ono et al. (31) did not observe the expression of E-cadherin in carcinomas of the samples analyzed. The lack of E-cadherin expression can be related to the process of formaldehyde fixation carried out for an extended time and the use of antibodies without species specificity. MMPs are endopeptidases that degrade the ECM. MMPs 2 and 9 degrade the denatured collagen and collagen IV, which integrates the basal membrane and is the first defense. As a result, the neoplastic epithelial cells cannot progress, invade, and metastasize.

Immunohistochemical staining of biomarkers is an important strategy for diagnosing tumors, determining the prognosis of lesions, and understanding the pathogenesis and malignant mechanism (11, 12, 22, 40, 41, 43). Dos Anjos (14) sought to evaluate the protein and gene expression of Bcl-2 and Bcl-2 associated X protein (BAX), which are the proliferative indexes in dogs with SCC treated with electrochemotherapy (ECT) as primary therapy. ECT was found to reduce tumor size and cellular proliferation. Further, the expression levels of Ki-67, BAX, and Bcl-2 did not significantly change with ECT treatment, suggesting that other proteins may play a role in the apoptosis pathways, carried out for an extended time.

SCC has a multifactorial etiology, including genetic predisposition, exposure to physical and chemical factors, exposure to ultraviolet light, papillomavirus infection, and changes in protein expression. Clinically, it can present with single or multiple lesions of variable size and the stages of the pathology differ between AK and SSC. Diagnosis is indispensable to distinguish between the stages of the disease and indication for the most appropriate treatment. Is still unclear the role of CPV infection in SCC development andidentification of viral CPV sequences by PCR is not sufficient to confirm the role of CPV in SCC development, since the virus could be present out of the cell nucleus, being not important as a carcinogenic factor. Therefore, positive immunohistochemistry or in situ hybridization showing CPV in nucleus of neoplastic cells can be considered the gold standard technique, which associates CPV with tumor development.

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