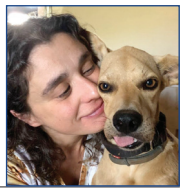
Exploring Key Biomarkers in Canine Cutaneous Squamous Cell Carcinoma



*C. Cardoso, R. Files, J. Prada, F. Silva and I. Pires**

Abstract

Squamous cell carcinoma (SCC) is one of the most common malignant skin tumours in dogs, significantly impacting canine health. Despite its prevalence, the study of biomarkers in canine SCC is still limited compared to human oncology. Biomarkers can provide valuable insights into the biological behaviour of tumours, aiding in diagnosis, prognosis, and treatment strategies. This review aims to consolidate the current knowledge on biomarkers in canine cutaneous SCC, highlighting parallels with human studies to enhance the understanding and potential clinical applications in veterinary oncology. Several key biomarkers have been identified in canine SCC, including cyclooxygenases (COX-2), intermediate filaments (vimentin), angiogenic factors (VEGF), and apoptosis-related proteins (survivin). Other biomarkers such as periostin, heat shock proteins, SOX9, E-cadherin, cytokeratins, galectins, and kallikreins have also been explored, providing insights into the pathophysiology and potential therapeutic targets for canine SCC. The expression patterns of these biomarkers often parallel those observed in human SCC, underscoring their relevance in both veterinary and comparative oncology. Identifying and characterising biomarkers in canine cutaneous SCC is crucial for advancing veterinary oncology. Continued research in this area could lead to improved diagnostic tools and targeted therapies, ultimately enhancing the management of SCC in dogs.

Key words: *cutaneous squamous cell carcinoma; biomarkers; cancer, canine*

Introduction

Cancer, characterised by the uncontrolled proliferation of cells, is a major cause of morbidity and mortality in humans and animals (Langsten et al., 2019).

Squamous cell carcinoma (SCC) is among the most frequent skin tumours in dogs, representing 3.9 to 10.4% of malignant skin tumours, similar to the rate of SCC in humans (Aguilera-Rojas et al., 2020; Ortloff et al., 2020a; Santana et al., 2016). SCC arises from the squamous epithelium and can occur in various locations, such as the skin, oral cavity, nasal cavity, tonsils, and lungs (Miyamoto et al., 2018).

Cutaneous SCCs in dogs are locally invasive with a low metastatic rate, similar to the human form, where metastasis primarily occurs in

Cláudia CARDOSO, DVM, MSc, CECAV, Rita FILES, MSc,; Justina PRADA, DVM, MSc, PhD, CECAV, Filipe SILVA, DVM, MSc, PhD, CECAV, Isabel PIRES*, DVM, MSc, PhD, CECAV, University of Tras-os-Montes and Alto Douro, Vila Real, Portugal (Corresponding author, e-mail: ipires@utad.pt)

locoregional lymph nodes (Dos Anjos et al., 2019). Cutaneous SCC in dogs can develop on any part of the skin, including the head, neck, abdomen, forelimbs, hindlimbs, perineum, and digits, but it typically exhibits slow growth (Nagamine et al., 2017). Despite the high incidence of these neoplasms in dogs, studies evaluating the inflammatory response in skin tumours are limited (Santana et al., 2016).

Tumour biomarkers are molecules produced by tumour cells that provide valuable information about the biological status of the tumour and can be used to assess disease progression and the effectiveness of therapeutic interventions (Lin et al., 2019). The number of established diagnostic tumour markers in veterinary is relatively small compared to human medicine. However, there is growing interest in researching biomarkers in veterinary medicine, which holds significant potential for advancing veterinary oncology (Estaller et al., 2021).

Biomarkers in oncology can be categorised into several groups, including those used for diagnosis, prognosis, treatment, and prevention. They play a critical role in identifying key mutations, molecular pathways, and other indicators that can guide personalised therapy and predict different outcome risks. Although veterinary medicine faces challenges in the widespread adoption of these tools, ongoing research aims to enhance their effectiveness, striving to achieve benefits comparable to those seen in human oncology (Aronson & Ferner, 2017; Ganguly et al., 2019; Adil et al., 2021; Alfano et al., 2023; Purkayastha et al., 2023).

This review aims to synthesise the existing research and explore future prospects concerning canine cutaneous SCC biomarkers.

Biomarkers in canine cutaneous SCC

Cyclooxygenases

Cyclooxygenases are enzymes responsible for catalysing the conversion of arachidonic acid into prostaglandins, with two primary isoforms identified: COX-1 and COX-2. COX-1 is generally involved in maintaining normal physiological functions, while COX-2 is an inducible enzyme predominantly associated with inflammatory processes (Gregório et al., 2021). COX-2 plays a critical role in the tumour microenvironment by promoting cancer stem cell-like properties, such as resistance to apoptosis, angiogenesis, inflammation, and enhanced invasion and metastasis (Millanta et al., 2016). This enzyme is expressed by tumour cell cancer-associated fibroblasts, and M2 macrophages in the tumour microenvironment. Its activity supports proliferative and survival signalling pathways, contributes to tumour hypoxia, and interacts with factors like YAP1 and anti-apoptotic mediators, thereby increasing resistance to chemotherapy (Musser et al., 2020).

COX-2 expression has gained attention due to its prognostic potential and implications for human and veterinary oncology therapeutic strategies. Numerous studies have explored COX-2 expression in dogs, particularly in relation to SCC. For instance, De Almeida et al. (2001) analysed 40 samples, 22.5% (nine cases) of which were cutaneous SCC, and reported positive immunoreactivity for COX-2 in all cases. Similar findings were noted by Bardagi et al. (2012) and Poggianni et al. (2012). In another study by Millanta et al. (2016), seven of eight canine cutaneous SCC samples tested positive for COX-2. Additionally, a study involving 37 cases of canine SCC found not only positive COX-2 immunoreactivity, but also a correlation with the histological grade of malignancy, underscoring its role in tumour progression (Luís et al., 2024).

Despite evidence of COX-2 expression in various canine tumours, the therapeutic potential of COX-2 inhibitors remains uncertain in many instances (Millanta et al., 2016). Tumours expressing COX-2 may respond to treatment with COX-2 inhibitors such as meloxicam or piroxicam, which have

shown potential in reducing cell proliferation and angiogenesis. In both human and veterinary oncology, the overexpression of COX-2 has been identified as a promising pharmacological target for preventing and treating several tumour types (Nardi et al., 2011; Eto et al., 2024; Luís et al., 2024).

Intermediate Filaments

Intermediate filaments are crucial components that provide structural integrity to cells and tissues. Among the six types of intermediate filaments, type III includes vimentin, desmin, glial fibrillary acidic protein, and peripherin. Vimentin, a 57-kD protein, plays a key role in maintaining cellular structure (Pieper et al., 2015). Vimentin is particularly significant in the context of epithelial-mesenchymal transition (EMT), a process essential for carcinoma progression, invasion, and metastasis (Nagamine et al., 2017; Jiang et al., 2020). During EMT, carcinomas typically exhibit increased expression of N-cadherin and vimentin, which can be detected through immunohistochemical analysis (Nagamine et al., 2017). In cutaneous squamous cell carcinomas, the upregulation of vimentin has been particularly noted in tumours undergoing EMT, establishing vimentin as a molecular marker for this process (Lan et al., 2014).

Several studies have explored the expression of vimentin in canine cutaneous SCC, confirming its role as a biomarker of EMT in this type of cancer. Pieper et al. (2015) examined vimentin expression in five samples of cutaneous SCC, finding that two showed positive vimentin expression. Nagamine et al. (2017) recorded vimentin positivity in all samples (58) of canine cutaneous SCC. These results indicate that vimentin expression in neoplastic cells is closely associated with the EMT process in canine cutaneous SCC, further solidifying its role as a biomarker of this transition in epithelial tumours (Pieper et al., 2015).

Angiogenesis biomarkers

Angiogenesis, the formation of new blood vessels, is a crucial process for the growth and metastasis of malignant tumours. This process is driven by various factors produced by tumour cells and the surrounding stroma, such as vascular endothelial growth factor (VEGF), which most contributes to tumour angiogenesis (Al-Dissi et al., 2007). VEGF facilitates the growth of new blood vessels that supply nutrients and oxygen to the tumour, thus supporting its expansion and the potential for metastasis (Hicklin and Ellis, 2005). VEGF has been widely studied in relation to cutaneous SCC in dogs, given its central role in tumour angiogenesis. Pioneering studies, such as Maiolino et al. (2000) showed positive immunostaining for VEGF in all 15 SCC samples evaluated, marking the importance of this factor in tumour development. Similar findings were reported by Dos Anjos et al. (2021) and Millanta et al. (2016), corroborating the relevance of VEGF in tumour aggressiveness.

Additionally, Al-Dissi et al. (2007) observed VEGF expression in 89% of analysed SCC samples, reinforcing the strong association between the expression of this factor and neoplastic growth. A more comprehensive study by Sozmen et al. (2020) evaluated VEGF and other angiogenic factors in SCCs from different anatomical locations. This study revealed that 80% of samples were positive for VEGF, in addition to high levels of other angiogenic mediators, such as basic fibroblast growth factor, platelet-derived growth factors (PDGF-A, PDGF-C and PDGF-α) and transforming growth factor-β1. In particular, SCCs from the nail bed showed greater expression of these factors.

These results confirm that VEGF and other angiogenic factors play crucial roles in the angiogenesis and aggressive behaviour of SCCs in dogs, providing important input for future therapeutic and prognostic approaches.

The high expression of VEGF in canine cutaneous SCCs highlights its relevance as a biological marker of tumour agressiveness. VEGF's ability to promote the formation of new blood vessels allows the tumour to maintain its growth, which is directly related to increased malignancy and metastatic potential. Strategies aimed at inhibiting VEGF activity can limit blood supply to the tumour, restricting its growth and spread. Given the essential role of VEGF in tumour angiogenesis, anti-angiogenic therapies represent a viable approach for treating cutaneous SCC in dogs, similar to what has already been explored in human and veterinary oncology (Maiolino et al., 2000; Al-Dissi et al., 2007; Millanta et al., 2016).

Survivin

Survivin is a protein with anti-apoptotic properties that plays a key role in developing and progressing various malignant diseases in humans. Studies indicate it is frequently expressed or overexpressed in canine SCC tissues (Miyamoto et al., 2018). Three studies analysed the expression of this protein in cutaneous SCC in dogs: Bongiovanni et al. (2009), Kavya et al. (2017) and Estaller et al. (2021).

The study conducted by Bongiovanni et al. (2009) included 19 samples of canine SCC, in which survivin expression was detected, with 100% of the samples showing nuclear expression and 84.2% cytoplasmic expression. Kavya et al. (2017) examined four cases of SCC among 23 epithelial tumours from a total sample of 40 skin tumours, identifying survivin gene expression in all cases, a finding also observed by Estaller et al. (2021) in their analysis of nine SCC samples. These studies indicate that survivin is widely expressed in canine cutaneous SCC and can potentially be used as a marker of malignancy.

In clinical studies with humans, high levels of survivin were associated with an unfavourable prognosis and a lower response to anticancer treatments (Estaller et al., 2021). Similarly, in dogs with SCC, high survivin expression is correlated with tumours with more aggressive behaviour, greater resistance to chemotherapy, and shorter survival time compared to tumours that do not have this protein (Kavya et al., 2017).

The anti-apoptotic function of survivin and its consistent expression in canine SCC highlight its potential as a prognostic biomarker. It can provide valuable information about the aggressiveness of the tumour and the likelihood of therapeutic success. In addition, the use of survivin as a target in therapeutic strategies may represent a new approach to improving the prognosis of dogs with SCC, increasing the effectiveness of treatments, and reducing tumour aggressiveness (Kavya et al., 2017).

Periostin

Periostin is a secretory protein that has recently been recognised as a member of the matricellular protein family. Its main function is related to the extracellular matrix, where it plays crucial roles in tissue remodelling and cell adhesion. Recent studies have demonstrated the participation of periostin in the pathophysiology of various conditions, including atopic dermatitis, asthma, inflammatory diseases, and cancer (Mineshige et al., 2018). In the oncological context, periostin has been associated with tumour development and progression processes, such as cell migration, invasion, and metastasis (Sonnenberg-Riethmacher et al., 2021).

Although research into the role of periostin in canine cutaneous SCC is still in its early stages, the available data points to its relevant role in disease pathogenesis. In a study involving 20 samples of canine SCC, Mineshige et al. (2018) reported intense periostin expression in the tumour stroma. These findings suggest that periostin, produced by fibroblasts in the stroma, may be involved in structural changes

and cell dynamics that promote tumour growth and progression (Mineshige et al., 2018). The expression of this protein in the stroma reinforces its possible contribution to creating a favourable environment for tumour development. The detection of periostin in the stroma of canine cutaneous SCC indicates its potential as a biomarker of the disease. As this protein is associated with tumour progression, it can serve as a prognostic marker, providing valuable information on tumour aggressiveness and the risk of metastasis. In addition, periostin's influence on the tumour microenvironment positions it as a promising therapeutic target. Interventions that inhibit periostin activity could compromise the stromal conditions that favour tumour growth and dissemination, limiting the progression of the disease in dogs (Mineshige et al., 2018). As research into periostin advances, it opens the possibility of developing new targeted therapeutic approaches that could significantly improve outcomes for dogs with SCC.

Heat Shock Proteins

Heat shock proteins (HSPs) are a family of highly conserved proteins first identified by Ritossa in 1962. Under normal conditions, HSPs are expressed at low levels, but their expression increases significantly in response to various cellular stresses, including hypoxia, oxidative stress, hyperthermia, radiation, exposure to ultraviolet light, and inflammation (Behdarvandy et al., 2020).

They play a fundamental role in maintaining cellular homeostasis under physiological conditions and during stress. They perform critical functions, acting as molecular chaperones, assisting in the proper folding of proteins, preventing protein aggregation, and aiding in the degradation of damaged proteins. They can exert anti-apoptotic or pro-apoptotic effects, thus influencing cell growth, differentiation, and cancer development. In particular, overexpression of Hsp72 has been observed in several human cancers, often associated with increased malignancy (Bongiovanni et al., 2009).

In veterinary medicine, HSPs are still in their infancy as biomarkers in canine cutaneous SCC. Two important studies have investigated the expression of HSPs in this context. Bongiovanni et al. (2008) studied the expression of the anti-apoptotic proteins Hsp27, Hsp72, and Hsp73 in canine SCC. They found that Hsp72 was more expressed in neoplastic epithelium than in normal tissue, with staining intensity ranging from moderate to strong. This suggests a potential role for Hsp72 in the progression of SCC. Similarly, Romanucci et al. (2005) examined the immunohistochemical expression and localisation of various HSPs in canine SCC, confirming that Hsp72 expression was generally more intense in neoplastic tissues compared to normal tissues.

Furthermore, the use of HSPs in therapeutic interventions could offer a new approach to the treatment of canine SCC. By inhibiting the protective functions of HSPs, it may be possible to increase the sensitivity of tumour cells to treatment, reduce their survival under stress, and limit tumour growth and spread. Continued research into the role of HSPs in canine SCC may lead to the development of new targeted therapies aimed at improving outcomes for affected dogs (Romanucci et al., 2005).

Sex-Determining Region Y-Box 9

Sex-Determining Region Y-Box 9 (SOX9) is an essential transcription factor in cell development and differentiation. This factor plays a critical role in several biological processes, including chondrogenesis, sex determination, and regulation of stem cell fate. As a transcription factor, SOX9 influences gene expression patterns that are fundamental for maintaining cellular identity and controlling important pathways in development (Lefebvre et al., 2019).

Recent research has identified an association between SOX9 expression and clinical outcomes in different types of cancer (Higo et al., 2018). In a study on human oral SCC, Sumita et al. (2018) reported that SOX9 was predominantly expressed in the nucleus of tumour cells, with some cases also showing cytoplasmic expression. These results suggest that SOX9 may play a role in tumour progression by influencing the behaviour of cancer cells and patient prognoses.

Currently, no specific studies on canine cutaneous SCC have evaluated the prognostic and therapeutic value of SOX9. However, a study by Fantinato et al. (2015) identified the presence of this molecule in all cases of SCC analysed, observing a greater intensity of labelling in most samples (Fantinato et al., 2015). These findings suggest that SOX9 may play a significant role in the progression of cutaneous SCC.

Furthermore, SOX9 has the potential to open new possibilities in the development of targeted therapies, contributing to the improvement of clinical outcomes in dogs affected by this type of tumour (Fantinato et al., 2015; Sumita et al., 2018).

E-cadherin and β-catenin

E-cadherin is a transmembrane protein in epithelial cells that plays a crucial role in cell adhesion and epithelial tissue development. It also functions as a tumour suppressor by sequestering excess cytoplasmic β-catenin (Gupta and Al-Dissi, 2023). The loss of adhesion molecules like E-cadherin, β-catenin, and desmoglein is associated with the development of invasiveness in tumour cells during carcinoma progression. These molecules are useful biological markers for evaluating prognosis in human carcinoma, including SCC (Nagamine et al., 2017). Reduced or absent E-cadherin expression is linked to poor differentiation, increased invasion, and metastatic behaviour in tumour cells (Lyakhovitsky et al., 2004).

The role of this molecule was also studied in canine SCC. Gupta and Al-Dissi (2023) investigated E-cadherin expression in different tumour grades of canine cutaneous SCC, correlating it with intratumoural microvascular density, proliferation index, and apoptotic index. Their results showed a negative correlation between E-cadherin scores and tumour grade, proliferation, and apoptosis. Similarly, Nagamine et al. (2017) found that lower expression of adhesion molecules correlated with individual morphological scores, including the degree of keratinisation, pattern of invasion, nuclear polymorphism, and number of mitoses. Another study analysed the expression of E-cadherin and found a correlation between reduced expression and tumour grade. Additionally, it was observed that E-cadherin in the cytoplasm might also play a role in tumour progression (Files et al., 2024). Thus, E-cadherin emerges as a promising molecule for the study of epithelial tumours and holds the potential for assessing tumour aggressiveness in prognostic studies.

Bongiovanni et al. (2011) reported reduced or absent membrane expression of β-catenin in nearly half of the cases of canine SCC studied, with cytoplasmic expression observed in all cases, suggesting that β-catenin may play a role in SCC progression in both dogs and humans.

Cytokeratins

Cytokeratins (CK) are intermediate filament proteins that play a crucial role in forming the cytoskeletal structure of epithelial cells. They are essential for maintaining cell integrity and are involved in various cellular processes such as division, differentiation, and signalling. CKs are classified into several types based on their physicochemical properties, and their expression patterns vary depending on the type and differentiation status of epithelial cells (Kok et al., 2018). In the context of cancer, CKs serve as

important biomarkers, and their detection through immunohistochemistry can assist in diagnosing and classifying tumours (Pieper et al., 2015).

Research on CK expression in canine cutaneous SCC has yielded valuable insights into the differentiation status and classification of these tumours. Kok et al. (2018) examined 110 samples of cutaneous epithelial tumours, including seven cases of cutaneous SCC. Their study assessed the expression of various CKs, finding that both well-differentiated and acantholytic SCCs were positive for CKAE1/AE3 and CK5/6. Additionally, CK16 and p63 showed positivity in some cases. Regarding CK19 expression, positivity was detected in only 54% of acantholytic SCC cases, highlighting variability in CK expression across different SCC subtypes (Kok et al., 2018).

In another study by Sanz Ressel et al. (2020a), which focused solely on the expression of p63 and CK5, homogeneous positive expression was observed in 72% of cases. These findings suggest that these two CKs may be valuable markers for identifying SCCs, particularly in cases where tumour type identification is challenging.

In a similar study by Pieper et al. (2015), the expression of CK14 and CK7 was evaluated. CK14 was positive in all SCC cases, while CK7 was negative. These findings highlight the importance of certain CKs, which may help aid the diagnosis of canine SCCs, highlighting the variation in expression between different types of tumours. As research advances, the role of CKs in the pathogenesis of SCC and their potential as therapeutic targets may become more evident, offering new perspectives to improve the treatment of canine cutaneous SCC (Pieper et al., 2015; Kok et al., 2018; Sanz Ressel et al., 2020a).

Galectins

Galectins are a family of β-galactoside-binding lectins that play significant roles in various normal and pathological processes, including immune response regulation, cell adhesion, apoptosis, and cancer progression (Kapucuoglu et al., 2009). Among the different types of galectins, Galectin-3 (Gal-3) has been widely studied for its involvement in cancer. Gal-3 can influence tumour behaviour by promoting cell proliferation, angiogenesis, and metastasis, making it a molecule of interest in cancer research.

The research by Marques et al. (2020) on the expression of Gal-3 in 50 samples of canine cutaneous SCC revealed that 56% of tumours showed low expression of Gal-3, while 44% showed high expression. However, the study concluded that Gal-3 is not a reliable prognostic marker for canine cutaneous SCC, as no significant differences were found in Gal-3 expression between the different histopathological grades of tumours. Despite the recognised role of galectin-3 in other types of cancer, its usefulness as a prognostic marker in canine cutaneous SCC appears to be limited (Marques et al., 2020).

Human Kallikreins

Human Kallikreins (KLKs) are a subgroup of 15 secreted serine proteases that play various roles in physiological processes, including skin desquamation, neural development, and the progression of certain cancers. These proteases are involved in the degradation of extracellular matrix components, modulation of cell signalling, and regulation of inflammation, all of which are important in maintaining normal tissue homeostasis. In the context of cancer, KLKs have been implicated in tumour growth, invasion, and metastasis, making them significant targets for cancer research (Filippou et al., 2016).

KLK5, a member of the KLK family, has been studied specifically for its role in cancer. Ortloff et al. (2020a) reported that KLK5 immunostaining was particularly strong in more differentiated cells exhibiting malignant phenotypes. The presence of KLK5

in these cells suggests that it may contribute to maintaining or promoting the malignant characteristics of the tumour. Furthermore, the study found that some KLK5-expressing cells were also positive for vimentin, a marker typically associated with epithelial-mesenchymal transition (EMT). The strong expression of KLK5 in more differentiated malignant cells and its coexpression with vimentin in certain cells suggest that KLK5 may be involved in the process of EMT, which is associated with increased invasiveness and metastatic potential in tumours. As such, KLK5 could serve as a potential biomarker to identify more aggressive forms of SCC, particularly those undergoing TMS (Ortloff et al., 2020b).

Furthermore, targeting KLK5 in therapeutic strategies could provide a new approach to the management of SCC. Inhibiting KLK5 activity may disrupt the processes that lead to tumour invasion and metastasis, potentially improving clinical outcomes for dogs with SCC. Further research is needed to fully understand the role of KLK5 in canine SCC and to explore its potential as a prognostic marker and therapeutic target (Ortloff et al., 2020b).

Cyclins

Cyclins are a group of essential proteins that play a critical role in regulating the cell cycle. They form complexes with cyclin-dependent kinases, which then phosphorylate target proteins to drive the cell cycle through its various phases. Cyclin A, in particular, is involved in both the S-phase, where DNA replication occurs, and the transition from the G2 phase to the M phase, which leads to cell division. Cyclin A ensures proper cell cycle progression by regulating these crucial checkpoints, and its dysregulation can lead to uncontrolled cell proliferation, a hallmark of cancer (Nozoe et al., 2002).

Cyclin A expression has been studied in several types of cancer, including SCC. In human oral SCC, increased Cyclin A expression has been associated with worse tumour grade, indicating a correlation between Cyclin A levels and tumour aggressiveness (Chen et al., 2003). Similarly, in veterinary medicine, Murakami et al. (2000) investigated the expression of Cyclin A in canine cutaneous SCC and found that it was frequently overexpressed. This overexpression suggests that Cyclin A may play a significant role in tumour cell proliferation, contributing to the aggressive nature of SCC in dogs. Elevated levels of Cyclin A may indicate faster cell cycle progression, leading to increased tumour growth and a potentially worse prognosis (Murakami et al., 2000).

In addition to its prognostic value, Cyclin A could also be explored as a therapeutic target. Given its critical role in cell cycle regulation, inhibition of Cyclin A function may slow or stop tumour cell proliferation, offering a new approach to cancer therapy. Further research is needed to fully understand the implications of Cyclin A overexpression in canine SCC and to explore its potential as both a prognostic marker and therapeutic target (Murakami et al., 2000; Chen et al., 2003).

Epidermal Growth Factor Receptor

Epidermal Growth Factor Receptor (EGFR) is a receptor tyrosine kinase that plays a crucial role in tissue development, regeneration, and homeostasis. EGFR involves cellular processes such as proliferation, differentiation, and survival. Under normal conditions, EGFR activation by its ligands triggers a cascade of downstream signalling pathways that regulate these vital cellular functions. However, abnormalities in EGFR, including overexpression or mutations, are often associated with tumorigenesis in various cancers. Dysregulated EGFR signalling can lead to uncontrolled cell growth, resistance to apoptosis, and other characteristics of malignancy, making it a significant factor in cancer development, including in canine SCC (De Lima et al., 2020).

Research highlights the crucial role of EGFR in the progression of canine cutaneous SCC. Sanz Ressel et al. (2020a) analysed 140 SCC samples and identified the presence of phosphorylated EGFR in 64.28% of cases, indicating that activation of this receptor is a common event in the malignant transformation of epidermal keratinocytes. EGFR activation was associated with more aggressive tumour behaviour and worse prognosis.

Another study by Luis et al. (2024) revealed that 43.2% of the samples showed overexpression of EGFR, while 56.8% showed low expression, identifying a correlation with the histological grade of malignancy. These findings reinforce the potential of EGFR as a prognostic marker to identify more aggressive tumours with worse responses to treatments (Luís et al., 2024).

Furthermore, EGFR represents a promising therapeutic target. Targeted therapies, such as tyrosine kinase inhibitors, have already shown efficacy in the treatment of human cancers with alterations in EGFR and can be explored in veterinary oncology to treat cases of canine SCC with EGFR overexpression or hyperactivation. Additional studies are needed to fully explore the therapeutic value of EGFR in this context (Sanz Ressel et al., 2020b; Luís et al., 2024).

Ki-67

Ki-67 is a nuclear protein that is closely associated with cellular proliferation. It is expressed during all active cell cycle phases, including the G1, S, G2, and M phases, but is absent in resting cells (G0 phase) (Sobecki et al., 2016; Remnant et al., 2021). Because of its specific presence in actively dividing cells, Ki-67 is a reliable marker for determining the growth fraction of a cell population. Its expression provides insight into the proliferation rate of cells, making it a valuable tool for assessing tumour aggressiveness in various cancers, including SCC (Sobecki et al., 2016; Remnant et al., 2021).

In canine cutaneous SCC, Ki-67 is frequently used as a proliferation marker to evaluate tumour growth and predict clinical outcomes. Studies have demonstrated that a high Ki-67 labelling index, indicating a higher proportion of actively proliferating cells, is often associated with more aggressive tumour behaviour. For instance, Luis et al. (2024) found that tumours with elevated Ki-67 expression tended to exhibit more rapid growth, increased invasiveness, and a greater likelihood of metastasis. These characteristics are typically linked to a poorer prognosis, making Ki-67 an essential marker for assessing the potential aggressiveness of SCC in dogs (Luís et al., 2024).

The Ki-67 labelling index is a powerful prognostic tool in canine cutaneous SCC, as it reflects the proliferative activity of the tumour. High Ki-67 levels are indicative of a fast-growing, aggressive tumour, which generally correlates with a worse prognosis. Evaluating Ki-67 expression can help veterinarians identify high-risk SCC cases that may require more aggressive treatment strategies (Luís et al., 2024).

In addition to its prognostic value, Ki-67 could potentially guide therapeutic decisions. Tumours with high proliferative activity, as indicated by Ki-67, might respond differently to certain therapies, particularly those targeting rapidly dividing cells. By incorporating Ki-67 assessment into the diagnostic and treatment planning process, it may be possible to tailor therapies more effectively to the specific characteristics of the tumour, thereby improving clinical outcomes for dogs with SCC (Sobecki et al., 2016; Remnant et al., 2021).

Other Biomarkers

Biomarker research in canine cutaneous SCC is an area in constant development, with efforts to identify new markers that can improve diagnosis, prognosis, and therapeutic approaches. The discovery of new biomarkers

is crucial to deepen the understanding of cancer progression, especially in veterinary medicine, where studies are still limited.

Among the markers that can be explored are Syndecans, epithelial-mesenchymal transition markers, and matrix metalloproteinases, all with relevant potential for understanding tumour biology. There are also many others we could mention.

Future research is essential to validate existing biomarkers and identify new ones, allowing for more informed clinical decisions and promoting important advances in treating and managing SCC in dogs.

Final considerations

Squamous cell carcinoma (SCC) in dogs is a prevalent and challenging condition that shares many similarities with its human counterpart, particularly in terms of its biological behaviour and the role of various biomarkers. This review highlights the current understanding of key biomarkers involved in canine cutaneous SCC, such as COX-2, vimentin, VEGF, and survivin. These biomarkers provide valuable insights into the tumour's biology and can potentially improve diagnostic accuracy, prognostic evaluations, and therapeutic strategies in veterinary medicine.

Despite the progress made in identifying these biomarkers, there remains a need for further research to better understand their implications in canine SCC and to develop standardised protocols for their use in clinical practice. Additionally, exploring the parallels between canine and human SCC can offer opportunities for comparative oncology, leading to advancements in treatment approaches for both species.

In conclusion, studying biomarkers in canine cutaneous SCC is essential for advancing veterinary oncology. Continued research and collaboration across disciplines will be vital in translating these findings into practical applications that can enhance the care and outcomes for dogs affected by this common and serious condition.

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Istraživanje ključnih biomarkera kod karcinoma skvamoznih stanica kože pasa

Cláudia CARDOSO, DVM, MSc, CECAV, Rita FILES, MSc, Justina PRADA, DVM, MSc, PhD, CECAV, Filipe SILVA, DVM, MSc, PhD, CECAV, Isabel PIRES*, DVM, MSc, PhD, CECAV, University of Tras-os-Montes and Alto Douro, Vila Real, Portugal

Karcinom skvamoznih stanica (SCC) jedan je od najčešćih malignih tumora kože pasa koji značajno utječe na zdravlje pasa. Unatoč njegovoj učestalosti, studija biomarkera SCC-a pasa i dalje je ograničena u usporedbi s ljudskom onkologijom. Biomarkeri mogu pružiti vrijedne uvide u biološko ponašanje tumora, doprinoseći dijagnozi, prognozi i strategijama liječenja. Ovaj pregled ima za cilj konsolidirati trenutna saznanja u svezi biomarkera SCC-a kože pasa, naglašavajući paralele s ljudskim studijama za bolje razumijevanje i potencijalne kliničke primjene u veterinarskoj onkologiji. Nekoliko ključnih biomarkera je identificirano u SCC-u pasa, uključujući ciklooksigenazu (COX-2), intramedijarne filamente (vimentin), čimbenike angiogeneze (VEGF) i apoptozne proteine (survivin). Ostali biomarkeri, poput periostina, proteina toplinskog šoka, SOX9, E-kadherina, citokeratina, galektina i kalikreina također su istraženi, osiguravajući uvide u patofiziologiju i potencijalne terapijske ciljeve za SCC pasa. Obrasci ekspresije tih biomarkera često su paralelni onima zamijećenima u SCC-u ljudi, naglašavajući njihovu važnost u veterinarskoj i komparativnoj onkologiji. Identifikacija i karakterizacija biomarkera SCC-a kože pasa ključni su za napredovanje veterinarske onkologije. Kontinuirano istraživanje na ovom polju moglo bi dovesti do boljih dijagnostičkih alata i ciljanih terapija, u konačnici poboljšavajući upravljanje SCC-om pasa.

Ključne riječi: *karcinom skvamoznih stanica kože, biomarkeri, tumor, psi*